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THE RELATIONSHIP OF THE BROMIDE CONTENT OF THE LUMBAR FLUID TO THAT OF THE CISTERNAL FLUID AFTER THE WALTER BROMIDE TEST

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During a recent study of the significance of the Walter bromide test in contrasted series of psychotic patients, occasion was taken to compare the bromide content of the cerebrospinal fluid obtained from the lumbar subarachnoid space with that of fluid nearly simultaneously aspirated from the basal cistern. It is the purpose of the present communication to analyze the results of this phase of the investigation and to comment briefly on the significance of certain of the conclusions reached.

MATERIAL AND METHODS. Hospital patients between 20 and 50 years of age were selected for this study. Our group of paretic patients consisted of individuals whose physical, serologic and spinal fluid findings were pathognomonic of paresis and who, despite an intensive course of anti-paretic treatment, had not improved sufficiently to justify parole. Our schizophrenic group included patients who had been diagnosed as such only after clinical observation lasting from four months to several years. According to the Walter technique, all the patients were given 0.01 gram of sodium bromide in aqueous solution by mouth per pound of body weight three times a day for five days. On the afternoon of the sixth day 5 cc. of cisternal fluid, 15 cc. of lumbar spinal fluid and 25 cc. of venous blood were removed, the order of removal of the samples of lumbar and cisternal fluid being reversed in half the cases as a further control. The bromide content of all samples was then determined by the original Walter technique and the results independently checked by the Toxopéus method (1).

[1] The author wishes to express his thanks to Dr. S. Katzenelbogen, in whose laboratory the bromide determinations were made, and to Dr. H. Goldsmith, physician-in-chief of the Psychopathic Hospital, who coöperated in making this study possible.

Statistical technique. For each series of observations the mean, its standard deviation (σ) and its probable error (P.E.) were calculated by standard statistical methods (2). In addition, the coefficient of correlation between the respective bromide contents of the lumbar and cisternal fluids was similarly obtained. The difference between any two comparable

TABLE 1

Differences and correlations between the respective bromide contents of the lumbar and cisternal fluid of 27 paretic and 22 schizophrenic patients after the Walter bromide test

SERIES NO.	SERIES	N	MEAN	σ (S.D.)$_m$	P.E.$_m$	SERIES COMPARED	DIFF.	P.E.$_d$	C.R.$_d$	CONCLUSIONS
1	P. Lf.	27	17.00	5.42	0.69	1–2	1.68	0.365	4.60	In the paretic series, the bromide content of the lumbar fluid after the Walter bromide test exceeds that of cisternal fluid by a mean difference of 1.68 ± 0.365 mgm. per 100 cc.
2	P. Cf.	25	15.32	2.49	0.336					
3	S. Lf.	21	13.36	3.88	0.57	3–4	1.43	0.148	9.65	In the schizophrenic series, the bromide content of the lumbar fluid exceeds that of the cisternal fluid by a mean difference of 1.43 ± 0.148 mgm. per 100 cc.
4	S. Cf.	22	11.93	3.38	0.485					
5	P. Bl.	26	48.37	11.51	1.53	6–5	1.55	2.52	0.61	No significant difference in the blood bromide content between the paretic and schizophrenic series after the Walter bromide test.
6	S. Bl.	22	46.82	13.91	2.00					
7	r_{lc}	47	0.980		0.093	7–8	0.980	0.135	10.5 7.7	Regression formulae: Lf. Bromides = 1.11 Cf. Bromides − 0.032
8	r_o	47	0.0		0.085					Cf. Bromides = 0.865 Lf. Bromides + 0.611

Bl.—blood; Br.—bromides; Cf.—cisternal fluid; C.R.$_d$—Critical ratio of the difference between comparable means; Diff.—difference between comparable means; Lf.—Lumbar spinal fluid; N—number of cases in a series; P.—paretic patients; P.E.$_m$—probable error of the mean; P.E.$_d$—probable error of the difference between comparable means; r_{lc}—coefficient of correlation between the respective bromide contents of the lumbar and cisternal fluids of the entire series; r_o—control coefficient of zero correlation; S.—schizophrenic patients; σ (S.D.)$_m$—standard deviation of the mean.

means was considered significant only when its critical ratio (Diff./ $P.E._d$) exceeded 3.0, or in other words, when the odds were greater than 22 to 1 that an actual difference inherent in the data of the series compared existed.

RESULTS. The significant statistical elaborations of the raw data are presented in table 1. From the latter analyses these conclusions become evident:

1. In the paretic group (series 1 and 2) the bromide content of the lumbar fluid exceeded that of the cisternal fluid by 1.68 ± 0.365 mgm. per 100 cc.

2. In the schizophrenic group (series 3 and 4) a similarly greater bromide content of the lumbar fluid was evident, the difference from the mean bromide content of the cisternal fluid in this series being 1.43 ± 0.148 mgm. per cent.

3. No significant difference in the blood bromide content of the two groups was demonstrated (series 5 and 6).

4. The actual amount of bromide in the cisternal fluid was closely related to that in the lumbar fluid, (series 7 and 8), the coefficient of correlation calculating to $+ 0.980 \pm 0.093$ and the regression formulae being:

a. Cisternal fluid bromides $= 0.865 \times$ lumbar fluid bromides $+ 0.611$

b. Lumbar fluid bromides $= 1.11$ cisternal fluid bromides $- 0.032$

COMMENT. The greater bromide content of the lumbar fluid in our series of cases with respect to that of the cisternal fluid evidently parallels the normal protein contents of the two fluids in the human, but is inverse to the corresponding sugar ratio (3). Interestingly, the difference in bromides between the two loci in schizophrenia was not significantly dissimilar to that observed in paresis (series 1 to 4). Again, the distribution of bromides had no apparent relationship to the physical activity of contrasted series of patients during the two hours preceding the double puncture. The relative constancy of the difference in the bromide content of the lumbar and cisternal fluids must therefore depend on:

1. A normal lack of free circulation of the cerebrospinal fluid between the basal cistern and the spinal subarachnoid space (4), and

2. A definitely greater ratio of dialysis or secretion to absorption of bromides in the lumbar region as compared to the cisternal.

Further, these conditions are evidently not influenced to a significant degree by the pathological changes in the central nervous system that occur in dementia paralytica.

SUMMARY

Twenty-eight paretic and 22 schizophrenic patients were given 0.01 gram of sodium bromide by mouth per pound of body weight thrice daily for five days, after which the bromide contents respectively of their blood, cisternal fluid and lumbar fluid were determined. A statistical analysis of the

data showed that the average bromide content of the lumbar fluid exceeded that of the cisternal fluid by a mean difference of 1.558 ± 0.119 mgm. per 100 cc., the difference applying without significant discrepancy both to the paretic and schizophrenic series. This phenomenon in both series apparently depends on: *a*, the normal absence of free circulation of the cerebrospinal fluid between the basal cistern and the lumbar space, and *b*, a difference in the ratios of secretion to absorption of bromides in the two loci.

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A COMPARISON OF THE ACTUAL AND THEORETICAL FORMS OF THE POST-INCISURAL PORTION OF THE AORTIC PULSE WAVE

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Frank (1899) deduced a mathematical expression for the diastolic portion of the aortic pulse wave, on the assumption that the outflow of blood from the aorta follows Poiseuille's law,

$$v = \frac{gr^4}{8l\eta} p \cdot t$$

where v is the quantity of blood leaving in t seconds, p the aortic pressure, η the blood viscosity, r and l the radius and length of a theoretical single tube of the same resistance as the vessels of the animal, and g the constant of gravity. The decrease in pressure is related to the decrease in volume of the blood in the aorta by the volume-elasticity coefficient, dP/dV, which may be regarded, as a first approximation, as a constant equal to E (Frank, 1920, 1930). The effective pressure, however, is really the difference between the pressure in the aorta, p, and the average of that in the peripheral capillaries or arterioles, p_r, into which the blood is flowing, a factor recognized by Cope (1911). Introducing this correction into Frank's equation and integrating, we have

$$\log \frac{(p - p_r)}{(p_0 - p_r)} = -\frac{E}{R} t,$$

where R stands for the constants of Poiseuille's equation, i.e., the resistance due to viscosity and the calibre of the vessels, and p_o is the pressure in the aorta when $t = 0$. This equation, if it could be shown to correspond with the facts, would be valuable, apart from theoretical considerations, in giving a quantitative measure of the peripheral resistance. The purpose of our experiments is to compare pressures calculated by this equation with those actually found.

METHODS. The aortic pressures were accurately determined under morphine-sodium barbital anesthesia with a calibrated optical manometer (Wiggers, 1928) inserted into the aorta through the left subclavian, or into

the innominate through the carotid. Fourteen dogs were used, the chest being open in 6 and intact in 8. In order to get a long diastolic curve the heart was stopped by stimulating the vagus. Pressures were determined at 0.2 second intervals.

RESULTS. The portions of the curve before and in the immediate neighborhood of the incisura were rejected, since during this period the flow in the first portion of the aorta is probably turbulent and Poiseuille's law would not hold. In the remainder of the curve we had no difficulty in showing in the vast majority of cases perfect correspondence between fact and theory. In the open chest experiments we obtained 60 curves; of these about two-thirds gave straight lines on plotting the logarithm of the pressure against time, i.e., $p_r = 0$. The experimental error of our measurements is about 3 mm. Hg; 90 per cent of the actual measurements differed from the theoretical curve by less than this. The remaining third

TABLE 1

A comparison of theoretical and calculated values for a typical curve

The constants used in calculation are $p_r = 0$; $p_o = 54$; $E/R = 1.45$

t	p FOUND	p CALCULATED
secs.	*mm. Hg*	*mm. Hg*
0	58	54
0.2	41	41
0.4	30	31
0.6	22	23
0.8	15	18
1.0	14	13
1.2	11	10

show a value of p_r greater than zero; when an appropriate value for this is inserted (a value which is quite definite, but which can be determined only by trial) the agreement is surprisingly close, the difference between found and calculated values averaging less than 1 mm. Hg. In three curves of this series, however, there are divergences which can be explained only by assuming that p_r falls during this period. (We have no cases in which it is necessary to assume a rise of p_r which would be improbable.) In the closed chest series of 155 curves, $p_r = 0$ in about one-fifth; about 10 per cent show a fall of p_r. The agreement between found and theoretical values is equally good. In none of the curves, even in the longest (of 11 sec. duration) is there any indication of a change in E/R during the curve; this makes it extremely improbable that either E or R varies with change of pressure. Changes of course occur from time to time as the resistance changes. Values for p_r are usually less than 40 mm. Hg, but may be greatly increased by the use of pressor drugs. The

agreement with theory is usually less close near the incisura, and breaks down completely, as one might expect, in the portion before it. The table shows a typical result, illustrating the close agreement in most of the figures with the greatest divergence near the incisura ($t = 0$).

SUMMARY

Frank's equation for the diastolic portion of the aortic pressure curve is slightly modified and shown to correspond very closely with the experimental facts. This affords a method for measuring the factors making up the peripheral resistance.

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THE RESPIRATORY METABOLISM OF ATROPHIC MUSCLE

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Few reports have been made relative to the respiratory metabolism of atrophic muscle. Langley and Itakagi (1917) reported the results of experiments on the oxygen consumption of atrophic muscle in vivo. More recently Hines, Leese and Knowlton (1931) have reported results concerning the respiratory quotient of intact denervated muscle.

This report is concerned with the results of experiments on isolated atrophic muscle from the rat with reference to the rates of oxygen consumption, glycogenolysis, lactic acid formation, and succino-dehydrase activity.

The oxygen consumption of a muscle is taken as a measure of its respiratory activity. A number of mechanisms other than the oxygen activating enzymes may become limiting factors in determining the rate of oxygen consumption. Among these are two general classes of enzyme systems: hydrolytic systems responsible for the cleavage of muscle substances with the ultimate formation of substrates for oxidation, and substrate activating systems whereby, in the presence of suitable oxidant systems, oxidation of substrate hydrogen is catalyzed. The rate of glycogenolysis and lactic acid formation was studied as a specific example of one of the first systems named, and the succino-dehydrase activity was used as an example of the second type of system mentioned above.

METHODS. *Denervation.* Rats were used as the experimental animals. The gastrocnemius muscle on one side was denervated by removing a section of the sciatic nerve at the level of the trochanter. The opposite muscle served as a control. These operations were performed under ether anesthesia.

Oxygen consumption. At designated times after denervation, animals were killed by a blow on the head and bled from the carotids. The muscle was removed from the animal and cut into strips as described by Richardson, Shorr, and Loebel (1930). Approximately one-tenth gram samples were weighed into a 2 cc. of phosphate buffered saline solution which was contained in tissue cups of about 20 cc. capacity. These operations usually occupied about 45 minutes' time. Provision was made for the absorption of CO_2 according to the method described by Dixon and Elliott (1930).

The cups were then attached to Warburg manometers and suspended and shaken in a constant temperature water bath at 37°C. Forty-five minutes were allowed for equilibration after which the manometers were adjusted to zero and the stopcocks closed. After 90 minutes the change in pressure was observed. The oxygen consumption was calculated in terms of cubic millimeters of oxygen used per gram of tissue (wet weight) per hour.

Large differences in the absolute values for oxygen consumption may result from differences in the time relationship of the death of the animal to the experimental observations. The oxygen consumption of isolated muscle strips invariably falls with time. The extent and rate of the decrease depends in part upon the preparation of the muscle strips. The gastrocnemius, because of the "herring bone" disposition of its fibers, can scarcely be stripped without considerable injury to the fibers, hence

TABLE 1

Average O_2 consumption during a 90 minute period in terms of cubic millimeters per gram muscle (wet weight) per hour

MUSCLE	NUMBER OF MUSCLES	OXYGEN CONSUMPTION	STANDARD DEVIATION	O_2 CONSUMPTION IN PER CENT OF NORMAL
Control	6	242	18	100
3 day atrophy	3	271	29	112
7 day atrophy	3	244	7	101
14 day atrophy	5	240	54	99
21 day atrophy	3	220	11	91
28 day atrophy	5	276	43	114
Control*	5	655	59	100
28 day atrophy*	3	705	21	108

* 30 minute period 45 minutes after removal of the muscle from the animal.

strips from this muscle seem to show a rather marked drop in the rate of oxygen consumption with time. Therefore, added determinations were made on control and 28 day atrophy muscles in which the measurements were concluded in a shorter time. By transferring the muscle strips directly to the tissue cups and allowing only 30 minutes for equilibration, the observations were begun within about 45 minutes after the death of the animal. The pressure change was noted at the end of 30 minutes, and the dry weight of the tissue in the cup determined. The values were calculated in terms of cubic millimeters of oxygen used per gram tissue per hour. (Wet weight, assuming 75 per cent water in muscle.)

The mean results obtained are given in table 1. Standard deviations are included as a measure of the variation about each mean.

Glycogen and lactic acid. Muscles intended for glycogen or lactic acid analysis were removed from the animal under amytal anesthesia. The

leg was skinned, and the muscle loosened so that it could be removed with two cuts of a sharp shears. The blood supply was disturbed as little as possible in this procedure. The skin was laid back over the muscle and five minutes allowed for recovery after which the muscle was quickly excised, care being taken to avoid stimulation. If to be analysed at once, the muscle was dropped into either ice cold 30 per cent potassium hydroxide or ice cold normal sulfuric acid depending on whether it was intended for glycogen or lactic acid determination. Muscles which were to autolyze were placed in Thunberg tubes, and the tubes evacuated at the water pump and placed in a constant temperature water bath at 37°C. for 30 minutes. At the end of this time the muscles were removed from the tubes and placed in ice cold alkali or acid as the analysis for which they were intended demanded.

Analyses were always made on pairs consisting of a normal and atrophic muscle from the same animal. The muscles were removed uniformly between 10 and 12 a.m. The rats used were all young fully grown males.

The glycogen was precipitated according to the method of Good, Kramer and Somogyi (1933) and reprecipitated according to the suggestion of Cori and Cori (1933). Hydrolysis of the glycogen was brought about by boiling in a water bath for 3 hours in presence of normal sulfuric acid. The glucose content of the hydrolysate was determined by the Shaffer-Hartman method. The results given in table 2 are expressed as the glucose equivalent in milligrams per 100 grams of tissue (wet weight).

The lactic acid determinations were made according to the method of Friedman, Contonio and Shaffer (1927). The results in table 3 are expressed as milligrams of lactic acid per 100 grams of tissue (wet weight).

Succino-dehydrase. Muscles for the determination of the succino-dehydrase activity were taken from animals which had been killed by a blow on the head and bled from the carotids. The succino-dehydrase was determined by one of the methods outlined below.

Method I. The muscle was weighed and ground with mortar and pestle with the aid of sand in the presence of 6 cc. M/15 Na-K phosphate buffer pH 7.4 per gram of tissue. The muscle brei was then transferred to a centrifuge tube and clarified by spinning at 18 revolutions per second for five minutes. The rate of centrifuging must be standardized if comparable results are to be obtained on different samples. High speed centrifuging definitely lowers the activity of an extract. The supernatant liquid was then decanted and allowed to stand for one-half hour at room temperature, after which its succino-dehydrase activity was measured by determining the times required for 0.2 cc. aliquots of the extracts to bring about the decoloration of 30 gamma of methylene blue with and without the addition of 0.2 cc. M/10 sodium succinate. The methylene blue was made up, in phosphate buffer solution, of such concentration

that 1 cc. contained 30 gamma of the dye. The whole reaction mixture was placed in a Thunberg tube which was evacuated at the water pump and suspended in a constant temperature water bath at 37°C. The activity was taken as the reciprocal of the decoloration time in minutes, and the difference in activities with and without added succinate was

TABLE 2

Glycogen (expressed as glucose) per 100 grams of muscle

MUSCLE	INITIAL			AUTOLYSIS*			DIFFERENCE
	Number of muscles	Glycogen	Standard deviation	Number of muscles	Glycogen	Standard deviation	Initial minus autolysis
		mgm.			*mgm.*		*mgm.*
Control	10	659	35	15	449	80	210
3 day atrophy	5	414	4	8	238	41	176
7 day atrophy	5	350	34	7	142	30	208
Differences on matched pairs; control minus 3 day atrophy	5	233	88	8	244	54	−11
Control minus 7 day atrophy	5	320	44	7	290	50	30

* 30 minute anaerobic autolysis at 37°C.

TABLE 3

Lactic acid content of muscles

Expressed in milligrams per 100 grams of tissue (wet weight)

MUSCLE	INITIAL			AUTOLYSIS*			DIFFERENCE
	Number of muscles	Lactic acid	Standard deviation	Number of muscles	Lactic acid	Standard deviation	Autolysis minus initial
Control	18	21	6	20	125	28	104
3 day atrophy	10	26	8	10	133	31	107
7 day atrophy	8	34	8	10	131	34	97
Differences on matched pairs; control minus 3 day atrophy	10	−7	4	10	−6	33	−1
Control minus 7 day atrophy	8	−10	5	10	−16	35	6

* 30 minute anaerobic autolysis at 37°C.

taken as a measure of the succino-dehydrase activity. The result was then transferred to milliequivalents of methylene blue decolorized per gram of tissue per hour.

Method II was the same as method I, except that Tyrode's solution was used in place of the phosphate buffer solution.

Method III was the same as method II except that the decoloration

time of 100 instead of 30 gamma of methylene blue was determined. The 100 gamme of dye were contained in a volume of 1 cc.

Method IV. The oxygen consumption of extracts, prepared as in method II, was determined by the Warburg technique. The extra oxygen consumption due to the addition of succinate was taken as a measure of the enzyme activity. Forty-five minutes were allowed for equilibration, and the oxygen consumption for the following thirty minutes was measured.

TABLE 4

Succino-dehydrase activity of phosphate buffer extracts of muscle

Activity expressed in terms of 1×10^{-2} milliequivalents of methylene blue decolorized per gram of tissue per hour.

DAYS AFTER DENERVATION	CONTROL MUSCLE			ATROPHIC MUSCLE			ATROPHY ACTIVITY*
	Number of muscles	Activity	Standard deviation	Number of muscles	Activity	Standard deviation	
7	7	1.9	0.6	7	1.0	0.5	53
14	6	1.2	0.3	6	0.8	0.1	67
21	6	1.6	0.3	6	0.9	0.3	56
28	11	1.4	0.5	11	0.9	0.3	64

* In per cent of normal.

TABLE 5

Succino-dehydrase activity of extracts of normal and of 7 day atrophic muscles

Activity expressed in terms of 1×10^{-2} milliequivalents of oxidant used per gram of tissue per hour.

METHOD	CONTROL MUSCLE			ATROPHIC MUSCLE			ATROPHY ACTIVITY*
	Number of muscles	Activity	Standard deviation	Number of muscles	Activity	Standard deviation	
I	7	1.9	0.6	7	1.0	0.5	53
II	13	2.6	0.4	6	1.4	0.3	54
III	6	3.6	0.2	6	1.9	0.3	53
IV	13	11.8	4.0	5	7.2	2.0	61

* In per cent of normal.

The results were calculated in terms of milliequivalents of oxygen used per gram of tissue per hour.

The above methods show two radical departures from the Thunberg method as described by Ahlgren (1925) in that an extract of unwashed tissue rather than minced washed tissue was used. Washing was dispensed with in these experiments because it was found that the activity of extracts made from washed muscle was appreciably lower than those prepared from unwashed muscle. The succinate concentration in fresh

tissue is quite low (Moyle, 1924), and the one-half hour allowed to elapse between the time of preparation of the extract and the determination of its succinodehydrase activity was sufficient to allow a considerable reduction in the amount of succinate originally present.

The results obtained are summarized in tables 4 and 5.

DISCUSSION. *Oxygen consumption.* Similar rates of oxygen consumption were found for control and atrophic muscles. The absolute values for oxygen consumption are definitely lower in the experiments where a longer time (1½ hours) elapsed after the death of the animal before measurements were started. The values where the observations were concluded sooner are in good agreement with a number of other reports concerning the oxygen consumption of normal muscle tissue (Stare and Elvehjem, 1933; Dye, 1933; Richardson, Shorr, and Loebel, 1930). It should be noted that the oxygen consumption rates by the two methods for the 28 day atrophic muscles are 108 and 114 per cent of the normal, indicating that the comparative values are much the same by either method. The comparison of these two rates also suggests that oxidative substrates and their precursors must occur in adequate amounts in both normal and denervated muscles.

It is known that a muscle undergoing atrophy following denervation loses chiefly cytoplasm; so that as the atrophy progresses the muscle comes to contain relatively more nuclei and connective tissue. The oxygen consumption of rats' connective tissue (tendon and fascia) was found to be of the order of 70 c.mm. per gram per hour, using the first method described above. Therefore, it would be expected that a relative increase in the amount of connective tissue would lead to a lower oxygen consumption. That such is not the case with these atrophic muscles may be due to the coincident increase of nuclei per unit weight. It would appear that the oxygen consumption rate of the nonconnective tissue portion of the muscle must actually increase as the atrophy progresses. Since the increase in the number of nuclei per unit weight is apparently directly proportional to the weight loss as is also the increase in connective tissue, it is suggested that any decreased rate due to the latter is approximately compensated for by an increased rate due to the nuclei. Therefore, any change in the oxygen consumption of the muscle as a whole remains within the limits of the experimental method used.

Langley and Itagaki (1917) reported the results of experiments which indicated an increase in the "in vivo" oxygen consumption of muscles undergoing atrophy following denervation. It must be remembered that the intact atrophic muscle shows fibrillary contractions which, other things being equal, would lead to a greater oxygen consumption per unit weight for the atrophic muscle than for the resting control muscle. This effect is removed in a study of the isolated muscle because the fibrillary

contractions disappear soon after the muscle is removed from the animal. The oxygen consumption rate of isolated muscle strips does not give a measure of the absolute rate of oxygen consumption by the intact resting muscle. By comparing two different isolated muscles, using the same technique and time relationships in each measurement, the relative oxygen consumption of the two muscles is probably an approximation to their relative resting rates when intact. Since these two types of muscle, control and atrophic, exhibit the same oxygen consumption rates "in vitro" it would follow, as Langley and Itagaki found, that the atrophic muscle, because of its fibrillary contractions, would have a greater oxygen utilization per unit weight "in vivo" than its homologous resting control.

Glycogen and lactic acid. The mechanism whereby glycogen is hydrolyzed to yield lactic acid has been further defined in recent years. (Lohmann, 1931; Milroy, Beattie, and Lyle, 1933.) It is known that under the influence of an enzyme system glycogen is hydrolyzed to give a hexose phosphate which is in turn further hydrolyzed to yield lactic acid. Whether there is but one intermediate and whether lactic acid is the only ultimate end product of this hydrolysis is not known. In these experiments we have measured the activity of two of the enzyme systems involved, the one promoting glycogen hydrolysis and the one catalyzing the shift from intermediate to lactic acid.

It will be seen (table 2) that the amount of glycogen disappearing during one-half hour anaerobic autolysis at 37°C. is the same, per unit weight, for normal and denervated muscle. This means that the glycogenase activity undergoes no change per unit weight of muscle during the first week of denervation atrophy. The same is true for lactic acid (table 3) which is produced in equal amounts by the two muscles during one-half hour anaerobic autolysis.

The atrophic muscles show a consistently lower glycogen content than their homologous controls. This is confirmatory of the results reported before (Hines and Knowlton, 1933) but is contradictory to practically all other published results on this subject. (Chandelon, 1876; Vay, 1894; Baum and Pichler, 1933.) It is suggested that the difference between our results and those of others may be due either to the use of a different species or to a difference in the method of taking the muscle from the animal.

The lactic acid content of the freshly excised denervated muscle is consistently higher than its control from the same animal. This may be due to the fibrillary activity exhibited by the atrophic muscle.

It seems from these results that the muscle undergoing atrophy of denervation has an undisturbed capacity for glycogenolysis and lactic acid formation during the first week of atrophy, in so far as the enzyme systems involved are concerned. However, the maximum amount of lactic acid that could be formed by atrophic muscle during anaerobic autolysis would

be less than that from control muscle because of the difference in glycogen content of the two muscles.

Succino-dehydrase. The extracts from the atrophic muscles showed a marked impairment in their ability to bring about the decoloration of methylene blue in the presence of sodium succinate (tables 4 and 5). The difference in the absolute levels of activity by methods I and II is due to the difference in the extraction fluids used. The difference between II and III, where the extraction fluid was the same, is due to the increased concentration of oxidant in method III. This type of effect has been described and discussed by Ahlgren (1925). The large difference between the absolute values by the methylene blue and oxygen consumption methods is probably due to the difference in the reduction potentials of the two oxidants, plus the fact that while the system in the Warburg technique is always saturated with oxidant such is not the case with the methylene blue method where the oxidant concentration keeps falling and approaches zero as the reaction proceeds. Of more significance for our purpose are the relative values of denervated to control activities. These show good agreement in the different techniques employed and indicate an early impairment in the activity of extractable succino-dehydrase of muscle following denervation. This finding stands in marked contrast to the unimpaired oxygen consumption and lactic acid formation of atrophic muscle. Furthermore, it furnishes an easily applicable test characterizing the atrophic muscle. Because of the meager amount of information available concerning the significance of muscle succinic acid metabolism, no functional significance can at present be given this finding.

SUMMARY

A study has been made of the oxygen consumption, glycogenolysis, lactic acid formation, and extractable succino-dehydrase activity, of rats' normal and atrophic gastrocnemii.

It was found that the rate of oxygen consumption of the atrophic muscles was the same as for control muscles. The significance of this finding in relation to the metabolism of intact atrophic muscle is discussed.

The glycogen content of the atrophic muscles was found to be definitely lower than for control muscles.

The amount of glycogen disappearing and lactic acid appearing during 30 minutes anaerobic autolysis was the same for the two types of muscle.

The initial lactic acid content of atrophic muscle was slightly, but definitely and invariably, greater than for control muscle from the same animal. This was probably a result of the fibrillary contractions of the denervated muscle.

The extractable succino-dehydrase activity of atrophic muscle was found to have fallen to about 55 per cent of the normal within one week after denervation.

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A QUANTITATIVE STUDY OF THE PRODUCTION OF SYMPATHIN

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Cannon and Rosenblueth (1933) obtained qualitative evidence which shows that sympathin—i.e., the sympathomimetic substance liberated on stimulating sympathetic nerves and responsible for local and remote responses (Cannon and Bacq, 1931)—is not the original mediator, adrenine or an adrenine-like product (Bacq, 1933), evoked directly by the sympathetic nerve impulses, but a compound of this mediator with the receptive substance present in the effector.

The present quantitative study of the production of sympathin has been carried out with the purpose of obtaining further evidence on the probable nature of sympathin and its relations to the adrenine-like mediator of sympathetic nerve impulses.

METHOD. Cats were used, under dial anesthesia. The nictitating membrane, denervated several days previously or acutely, was selected as an indicator for sympathin (Rosenblueth and Cannon, 1932). The sources of sympathin were the effectors supplied by the internal branch of the right stellate ganglion (mainly the heart), by the right splanchnics after removal of the adrenals (liver and gastro-intestinal tract) and by the nerves surrounding the duodenohepatic artery (mainly the liver); thus, chiefly sympathin E was obtained (Cannon and Rosenblueth, 1933). Cocaine was frequently employed to enhance the responses of the nictitating membrane (Rosenblueth and Cannon, 1932).

Short (1 to 3σ) rectangular electric waves from a multivibrator (see Rosenblueth, 1932b) were applied at variable frequencies to the nerves, crushed or severed centrally. Relatively strong shocks were employed to insure an activation of all the fibers in the nerve stimulated, so as to deal exclusively with temporal variations in the stimuli. Even when the heart or the liver was stimulated the adrenal glands were generally ligated. A possible spread of the strong shocks used, when stimulating the cardio-accelerators, to the neighboring right vagus was eliminated by previous section of this nerve in the neck at the time when the superior cervical sympathetic ganglion was removed.

The isotonic contractions of the nictitating membrane were recorded on a kymograph by means of a lever adjusted to furnish about a 15-fold

magnification. The maximal height of the tracing was measured for each response. The results are therefore expressed in conventional units.

RESULTS. A. *Comparison of the responses of the nictitating membrane to stimulation of its nerve supply and to adrenalin.* As will be shown in the discussion, this comparison of the effects of sympathin produced locally with those of adrenine is indispensable for interpreting the results from sympathin produced elsewhere, to be described below. It has been reported previously that both the responses to variable doses of adrenalin (Rosenblueth, 1932a) and to variable frequencies of stimulation of the nerve supply (Rosenblueth, 1932b) yield rectangular hyperbolas. The issue to be determined here was whether, in a given preparation and after choosing suitable units for the frequencies and the doses of adrenalin, all the responses obtained from the two types of excitation would fall on the same hyperbola—i.e., whether a linear transformation between the frequency and the doses would result in a single curve.

That a single curve will fit the responses to the two sets of stimuli is shown in figure 1. After dial and cocaine a series of responses to diverse frequencies of maximal stimulation of the cervical sympathetic was recorded. The maximal heights of the responses are plotted in figure 1 as dots. Various doses of adrenalin were then injected intravenously in the same cat; the corresponding responses are plotted as circles. The large doses of adrenalin evoked eye movements in the animal, which explain the scattering in this region of the curve. The fit is, however, very satisfactory and the deviations are quite unsystematic.

B. *The output of sympathin as a function of the frequency of stimulation.* When any one of the sources of sympathin mentioned under "Method" was stimulated by maximal shocks applied at increasing frequencies, the following results were obtained. Low frequencies did not elicit any contraction of the denervated nictitating membrane. At a critical threshold frequency the responses of the membrane appeared and were then greater as the frequency increased. Figures 2 and 3 illustrate typical results for the cardio-accelerators and the hepatic nerves, respectively.

There was usually a continuous relative decrease of the output of sympathin, as the stimulus applied later in the series yielded generally a smaller response than earlier. In a few instances, however, stable preparations occurred, in which the original responses were fairly reproducible after a long series of stimuli. These cases were deemed of particular significance for judging the quantitative relations between the output of sympathin and the frequency of stimulation.

In all cases, whether progressively faster, progressively slower, or random frequencies were applied, the general features of the curves reproduced in figures 2 and 3 appeared consistently. We would emphasize two features which distinguish these curves from those obtained by variable doses

of adrenalin: the threshold, already mentioned, and the fact that the calculated asymptote which they approach at high frequencies is different from that toward which the responses from adrenalin tend. This asymptote, which will be considered further in the mathematical discussion, may be lower (figs. 2 and 3) or higher (fig. 4) than that for adrenalin.

The frequent progressive decrease of the output of sympathin was usually associated with a progressive increase of the threshold frequency.

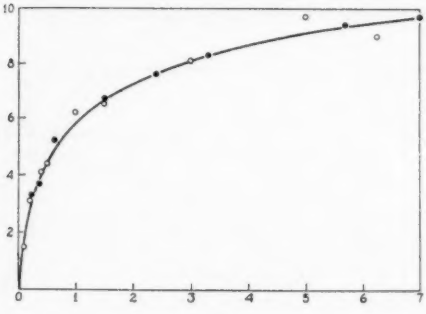

Fig. 1

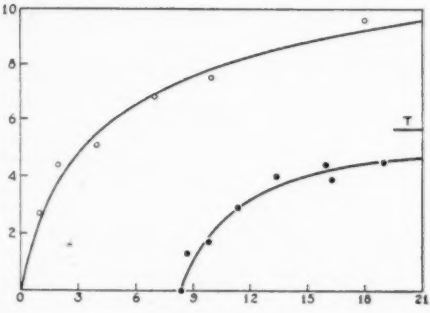

Fig. 2

Fig. 1. Dial; cocaine. Ordinates: isotonic responses of the nictitating membrane (cm. in the record). Abscissae: amounts of adrenalin injected intravenously (unit: 0.02 mgm.) and number of maximal shocks applied per second to the cervical sympathetic. The circles indicate the responses to adrenalin; the dots, to stimulation of the nerve supply.

Fig. 2. Right cervical sympathetic and right vagus sectioned 6 days previously. Dial; cocaine. Ordinates: isotonic responses of the right nictitating membrane (cm. in record). Abscissae: amounts of adrenalin injected intravenously (unit: 0.0004 mgm.) and number of maximal shocks applied per second to the right cardio-accelerators. The circles indicate the responses to adrenalin; the dots, to stimulation of the cardio-accelerators. The dot which marks the threshold frequency and the horizontal line, *T*, which marks the asymptote of the lower sympathin curve were calculated as described in the section on mathematical analysis. The order in which the different frequencies of stimulation of the cardio-accelerators were carried out was the following: 16, 9.8, 11.4, 8.7, 13.4, 16.3 and 19.

For this reason, and because of the steepness of the curve at the initial ascending part, this region is apt to show greater scattering of the results.

The threshold frequency varied considerably from one preparation to another. It was usually lower for the splanchnic or hepatic nerves (3 per second, average of 7 experiments) than for the cardio-accelerators (8 per second, average of 7 experiments). It was found to depend on the sensitivity of the nictitating membrane, being as a rule lower in the preparations in which the membrane had been previously denervated postganglionically than in those in which acute denervation and cocaine were used.

Cocaine reduces the threshold frequency, however, independently of its effects on the membrane, for in chronically denervated preparations the injection of cocaine may have a negligible effect on the sensitivity of the membrane, as judged by the responses to adrenalin, while it significantly lessens the threshold frequency for production of effective sympathin (fig. 5).

The horizontal asymptote (calculated, see Mathematical Analysis) is likewise dependent on the sensitivity of the nictitating membrane, being usually higher than that for adrenalin in chronically denervated animals

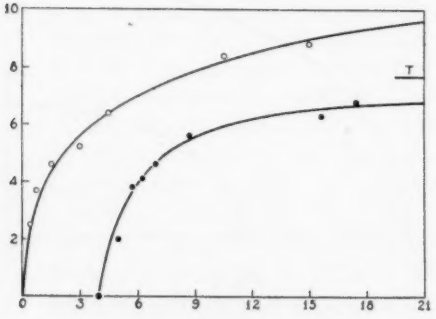

Fig. 3

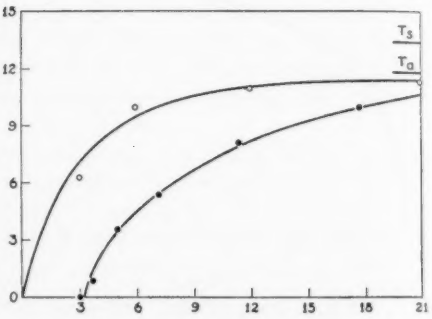

Fig. 4

Fig. 3. As in figure 2, but stimulation of the right splanchnics after removal of the adrenals. The order in which the different frequencies of stimulation were applied was the following: 5, 7, 8.7, 6.3, 17.5, 5.8 and 15.7. The unit for the doses of adrenalin injected is 0.006 mgm.

Fig. 4. Nictitating membrane denervated by removal of the superior cervical sympathetic ganglion 8 days previously. Dial. Ordinates: responses of the membrane (cm. in record). Abscissae: frequencies of stimulation of hepatic nerves and doses of adrenalin (unit 0.00013 mgm.) injected intravenously. Upper curve (circles): adrenalin. Lower curve (dots): sympathin. The lines T_a and T_s mark the calculated asymptotes for adrenalin and sympathin, respectively, higher for sympathin than for adrenalin in this animal.

injected with cocaine (fig. 4), approximately similar to that for adrenalin in chronically denervated animals without cocaine and being lower than that for adrenalin (in some instances only 30 per cent) in the cases of chronic or acute preganglionic decentralization and cocaine (figs. 2 and 3). Cocaine may raise the asymptote although it may not alter the sensitivity of the membrane, an effect similar to that which was pointed out above when the reduction of the threshold frequency was discussed (fig. 5).

C. *The summation of the effects of sympathin obtained from different sources.* For these experiments electrodes were placed on both the right cardio-accelerators and the right splanchnics after removal of the adrenal

glands. Each of the nerves was stimulated separately and the responses of the denervated membrane recorded; the two nerves were then stimulated simultaneously with the same stimuli; finally separate stimulation was again applied. The cardio-accelerators were usually excited by means of a Harvard inductorium while the splanchnics were brought into action with

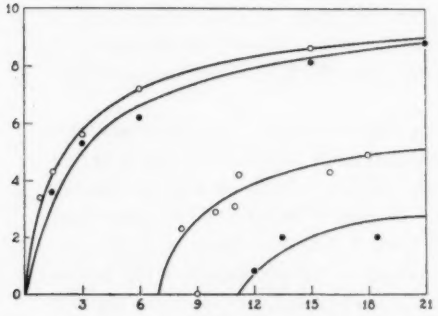

Fig. 5

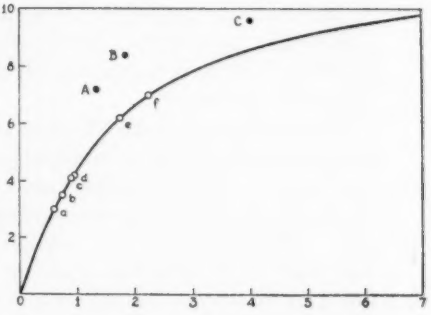

Fig. 6

Fig. 5. Effects of cocaine on the responses of the nictitating membrane to adrenalin and to sympathin. Membrane denervated by removal of the superior cervical sympathetic ganglion 6 days previously. Dial. Ordinates: responses of the membrane in centimeters in record. Abscissae: frequencies of stimulation of the right cardio-accelerators and doses of adrenalin (unit 0.00013 mgm.) injected intravenously. The two upper curves represent the responses to adrenalin, the two lower curves the responses to sympathin. The responses plotted by dots were obtained before, those plotted by circles after, injecting 8 mgm. cocaine hydrochloride per kilogram. The order in which the different frequencies of stimulation were applied after cocaine was the following: 8.2, 11.2, 16, 10, 11, 9, 18.

Fig. 6. Right superior cervical sympathetic ganglion removed 6 days previously. Dial; cocaine; adrenals ligated. Ordinates: isotonic responses of the right nictitating membrane (cm. in record). Abscissae: amounts of adrenalin,—unit 0.0004 mgm. The curve drawn is that obtained from 5 injections of adrenalin; the corresponding points do not appear in the figure. Points *a*, *d* and *f* are the responses to stimulation of the cardio-accelerators and points *b*, *c* and *e*, to stimulation of the right splanchnics, separately. They are plotted on the curve at the corresponding ordinates, thus quantifying the sympathin in terms of adrenalin. Points A, B and C are the responses to simultaneous stimulation of the two sets of nerves with the same stimuli used in the separate stimulations, as follows: $A = a + b$, $B = c + d$ and $C = e + f$. A, B and C are plotted at the abscissae obtained by adding those of the separate stimuli. For explanation see text.

the multivibrator. A curve for the contractions to adrenalin was obtained in the same animal, and the responses to sympathin were compared with this curve.

The magnification of the recording lever used was such that the maximal contractions of the membranes were as a rule from 10 to 12 cm. in the rec-

ord. When each of the stimuli was set so as to furnish separately small responses (about 2 cm.), simultaneous stimulation yielded generally a summation greater than linear (more than 4 cm., up to 7 cm.). Medium-sized individual responses (about 4 cm.) evoked an approximately linear summation (about 8 cm.). Higher individual responses (over 6 cm.) resulted in a summation less than linear (about 9 cm.). This latter response to simultaneous application of strong and frequent stimuli, however, was higher than that which would have occurred from a dose of adrenalin equal to the sum of two doses that would match the effects of the separate stimulations of the nerves.

The summation of the effects of sympathin from two sources, therefore, exceeds the effects which would be predicted from a quantification in terms of adrenine. The excess decreases as the individual amounts of sympathin employed in the summation increase. Figure 6 illustrates the phenomenon. The hyperbola is that obtained from 5 different doses of adrenalin. The points a, d, f, and b, c, e, are the average of the responses of the membrane to separate stimulations of the cardio-accelerators and the hepatic nerves, respectively, before and after the simultaneous stimulations. The points A, B and C are the responses to the simultaneous stimulations, their ordinates are the heights of these responses and their abscissae are the sums of the abscissae of the corresponding separate stimulations: $A = a + b$; $B = c + d$; $C = e + f$.

Discussion. From the data reported in section A (fig. 1) we may conclude that the amounts of the adrenine-like mediator M liberated by sympathetic nerve impulses are a linear function of the frequency F of stimulation, i.e., $(M) = kF$. This relation had been assumed previously (Rosenblueth, 1932b); it here finds an experimental corroboration.

If, then, sympathin were M, i.e., adrenine, one would infer that the responses of the nictitating membrane to variable doses of sympathin should yield a curve similar to that obtained from variable doses of adrenalin. This curve should have a horizontal asymptote corresponding to the maximal response from the nictitating membrane, i.e., the same asymptote as that for adrenalin.

In connection with this inference it is interesting to recall that the amounts of adrenine secreted by the adrenal gland are directly proportional to the frequencies of stimulation of its nerve supply and that a linear transformation of the abscissae yields therefore a single curve for the responses to secreted adrenine and to injected adrenalin (Rosenblueth, 1932b).

The inference, however, is not fulfilled experimentally. The asymptote for sympathin may be higher or lower than that for adrenine (figs. 2, 3 and 4). Furthermore, cocaine or denervation, either of which has a negligible effect on the maximal response of the membrane to adrenine, raises strikingly the maximal response to sympathin (section B, fig. 5). This leads us

to conclude that sympathin is not adrenine and that the asymptote in the curves for sympathin is not dependent on the capacity of the membrane to contract but on the capacity of the stimulated organs to yield sympathin.

In addition, if sympathin were adrenine the summations of effects on simultaneous stimulation of two sources should fall on the adrenine curve. That is, if the response evoked by the sympathin S_1 obtained from source 1 is equal to that elicited by a dose of adrenine A_1, and the contraction of the membrane evoked by the amount of sympathin S_2 obtained from source 2 is equal to that produced by a dose of adrenine A_2, then, if sympathin were adrenine, the response to $S_1 + S_2$ should be equal to that of $A_1 + A_2$. This is not the case, however, as reported in section C (fig. 6). The combination $S_1 + S_2$ evokes consistently a larger response than that obtained from $A_1 + A_2$. We must again conclude that sympathin is not adrenine.

The hypothesis suggested by Cannon and Rosenblueth (1933), that sympathin is not the adrenine-like mediator, but a compound of this mediator with the receptive substance present in the source, leads to the following inferences in regard to the evidence presented in this paper. The amounts of sympathin obtainable from a given source, when the amounts of the mediator (M) are increased by the frequency of stimulation, will be limited by the quantity of the receptive substance present. If the responses of the membrane bear a linear proportion to the amounts of sympathin (Rosenblueth, 1932b), these responses will be likewise limited by the quantity of receptive substance in the source, not in the membrane. The horizontal asymptote of the contractions of the membrane to sympathin may therefore be different from that of the responses to adrenine. This inference is confirmed by the experiments (section B).

Since the responses of the nictitating membrane are now assumed to be in linear proportion to the amounts of sympathin produced, the addition of two given amounts should lead to linear summation of the responses. This inference is not fulfilled by the experimental results; with small doses of sympathin the summation of the responses is greater than linear, and with large doses smaller (section C, fig. 6). Before an explanation for this discrepancy is attempted it is convenient to discuss the threshold frequency of stimulation necessary for the sympathin effects to appear (figs. 2, 3 and 4).

This threshold implies that a certain concentration of sympathin is required before it can reach the nictitating membrane and show its effects thereon. The critical concentration may be either at the source, or in the blood, or in both. We can easily conceive that if the rate of destruction and utilization of sympathin at the source equals the rate of production, there will not be any available for diffusion into the blood stream. That beyond a certain frequency the rate of production becomes greater than that of destruction is shown by the fact emphasized previously (Rosen-

blueth, 1932b) that equilibrium of contraction (a plateau) is obtainable only at low frequencies (up to about 2 per second for the nictitating membrane); higher frequencies elicit throughout the period of stimulation a continuous slow rise, steeper the higher the frequency employed (see figs. 2 and 3, *loc. cit.*). It is probable that diffusion outside the muscle stimulated begins at frequencies where equilibrium is not maintained.

On the other hand, it is likewise probable that sympathin poured into the blood stream will not reach the nictitating membrane, now employed as an indicator, until the quantity present in the blood is sufficient to permit some to pass beyond the cells which are first accessible, i.e., the smooth muscle of the blood vessels might take up completely a small quantity of sympathin produced at a remote point in the body, and therefore might prevent any from reaching the membrane. We can conceive the intervening vascular course as a dead space which has to be filled before the observed structures lying beyond it are reached by the activating substance.

If this "dead space" exists for sympathin it probably likewise exists for injected adrenalin. The curves for variable doses of the latter should then not pass through the origin, but should show a threshold. This apparent threshold does exist, as was pointed out previously (Rosenblueth, 1932a), but is for adrenine extremely small when compared with the range of effective doses, and can therefore be neglected.

That the dead-space mechanism probably plays a significant rôle in the results reported here is indicated by the satisfactory explanation which it furnishes for the excess in the summation of small doses of sympathin (section C). Separate stimulation of each of the two sources S_1 and S_2 furnishes then sympathin for the dead space (S^D) and for the membrane (S^R). Simultaneous stimulation then gives $S_1^D + S_1^R + S_2^D + S_2^R$, where $S_1^D = S_2^D = S^D$. The response of the membrane is then that corresponding to $S^D + S_1^R + S_2^R > S_1^R + S_2^R$. The term S^D may then explain the greater than linear summation.

As regards the less than linear summation of the larger doses of sympathin (section C) two explanations are possible, either or both of which may be important. First, the output of sympathin from a source depends not only on the concentration of sympathin inside the source, but also on the concentration in the blood stream, for the passage of sympathin from the source into the blood is due to a process of diffusion and the gradient of concentrations will determine this diffusion. It is then readily conceivable that the sympathin in the blood coming from a first source may interfere with the output of a second source, and *vice versa*, and that this interference will be greater for larger than for smaller doses.

The second explanation is the following. In the circulating blood sympathin may not be exclusively the compound MH of the mediator M with the receptive substance H, but there may be also some M that has diffused

out. If this be so, there would be at higher frequencies of stimulation, because of the law of mass action, relatively higher concentrations of M than of MH, and hence also relatively a greater diffusion of M than of MH. In such a mixture M, assumed to be equivalent to adrenine, would tend to make the summation less than linear (see curves for adrenine in all figures).

If the latter explanation holds, i.e., if circulating sympathin = M + MH, the failure of sympathin E (Cannon and Rosenblueth, 1933) to relax the non-pregnant uterus of the cat may be accounted for as follows. If pure M (adrenine) reaches a smooth muscle with the I receptor, MI is formed and relaxation ensues. If ME enters the I cells, however, it may dissociate, and the free M may combine with I so that equal amounts of MI and ME are ultimately present and the elastic properties of the muscle do not change. A similar situation would ensue if a small amount of M were added to the ME. If, finally, equal amounts of ME and MI are given (e.g., by stimulation of the splanchnics after ligation of the adrenals and severance of the nerves to the liver), then either ME or MI will preponderate at the indicator, according to whether it possesses the E or the I receptor, and contraction or relaxation, respectively, will occur. In connection with these views it is interesting that there appears frequently a slight initial and delayed contraction of the uterus when predominantly E sources are stimulated (see stimulation of the cardio-accelerators, Cannon and Rosenblueth, *loc. cit.;* the effect has been confirmed in unpublished experiments with Z. M. Bacq).

Whether sympathin be pure MH or MH + M, a combination of the mediator with the receptive substance is involved—i.e., a chemical reaction occurs before the excitatory substance which determines the responses is produced. The hyperbolas obtained from excitation of autonomic effectors by adrenine (Rosenblueth, 1932a) or nerve impulses (1932b), were interpreted as due to a chemical reaction. This reaction could have been present at any of the functionally related steps which lead to the specific response of the effector, e.g., it could be a step in the effector system proper, not related to the excitatory process but to the response. The evidence presented here is in favor of the view adopted (*loc. cit.*), that the formation of the excitatory substance involves a chemical reaction.

The effects of cocaine (section B) can be accounted for in the following manner. If, as suggested previously (Rosenblueth, 1932a), cocaine increases the permeability of smooth-muscle cells to adrenine and sympathin and diminishes the rate of their destruction, then its administration will not only favor the penetration of the substances into the indicator, but it will favor also the outward diffusion of sympathin from the source, thus lowering the threshold frequency and raising the horizontal asymptote of the corresponding curves (fig. 5).

The explanation of the decline of the responses on successive stimulations in some animals (section B) requires further data because of the complexity of the variables possibly involved.

Mathematical analysis. Several simplifying assumptions have to be introduced in order to make a mathematical formulation of the results. The analysis, however, is of importance in that it permits a precise definition of the significant variables, and in that it furnishes a probably sufficiently accurate means of determining the asymptotes of the curves, which, as shown in the preceding discussion, have considerable theoretical interest.

As has been shown previously (Rosenblueth, 1932a), the responses (R) of the nictitating membrane to adrenine (M) yield a hyperbola of the formula

$$R_m = \frac{M}{k + k'M} \quad \ldots\ldots (1)$$

If sympathin (S) is MH, the responses of the membrane (i) to sympathin bear a linear relation to the amounts of sympathin (Rosenblueth, 1932b),

$$R_s = k''S_i \quad \ldots\ldots (2)$$

The amounts of sympathin produced at the source (S_s) will be (Rosenblueth, 1932b)

$$S_s = \frac{F}{a + bF} \quad \ldots\ldots (3)$$

Let us now assume that the amounts of sympathin which reach the nictitating membrane used as an indicator (S_i) are a linear function of the amounts of sympathin produced at the source, i.e.,

$$S_i = \alpha (S_s - \theta) \quad \ldots\ldots (4)$$

where Θ stands for a threshold (see p. 216) assumed to be constant. We then have, after the proper substitutions,

$$R_s = \frac{F - m}{n + pF}$$

which gives the responses of the membrane in terms of the frequency of stimulation of the source. This last formula represents a rectangular hyperbola which crosses the F axis at the point m and whose horizontal asymptote is $1/p$. That this formula fits the experimental data satisfactorily is shown by the following tests applied to the observations illustrated by the lower curves of figures 2 and 3.

Figure 2

Calculated $m = 8.4$; $n = -1.08$; $p = 0.181$

F	CALCULATED R	OBSERVED R
8.7	0.61	1.3
9.8	2.03	1.7
11.4	3.06	2.9
13.4	3.73	4.0
16.0	4.19	4.4
16.3	4.23	3.9
19.0	4.50	4.5

Figure 3

Calculated $m = 4$; $n = -0.237$; $p = 0.13$

F	CALCULATED R	OBSERVED R
5.0	2.44	2.0
5.8	3.51	3.8
6.3	3.98	4.1
7.0	4.50	4.6
8.7	5.30	5.6
15.7	6.52	6.3
17.5	6.67	6.8

The fit is further emphasized by the satisfactory value of the calculated m in the two instances.

Only the series of observations obtained from relatively stable preparations were tested. The preparations showing a marked persistent decrease of the responses (p. 210) were discarded from this standpoint. All the cases tried yielded satisfactory tests. In a few instances $p = k'$, i.e., the maximal responses from either adrenine $(1/k')$ or sympathin $(1/p)$ were approximately equal. Usually $p \neq k'$ (section B).

If the effects of cocaine were those suggested above (p. 217) the following changes would occur: $\Theta_c < \Theta_w$; $A_c < A_w$; where the subscripts w and c denote the preparation *without* and *after* cocaine. Hence $M_c < M_w$; $N_c < N_w$; $p_c < p_w$. Furthermore, $-N_c/p_c < -N_w/p_w$. Therefore, a lower threshold, a higher horizontal asymptote and a curve steeper at the start should obtain after cocaine. These changes were found experimentally (fig. 5).

SUMMARY

With the nictitating membrane as an indicator for sympathin and adrenalin, variable frequencies of maximal stimulation were applied to the cervical sympathetic, the cardio-accelerator and the hepatic nerves sepa-

rately or simultaneously and the responses thus obtained were compared with those elicited by variable doses of adrenalin.

The results obtained were the following. The responses to adrenalin and to cervical sympathetic nerve impulses yield a single curve (fig. 1). The responses to adrenalin and those to sympathin from the heart or the liver yield different curves (figs. 2, 3 and 4). Finally, the responses to simultaneous stimulation of the cardio-accelerators and the hepatic nerves are greater than would be expected if the sympathin obtained from separate stimulations is quantified in terms of adrenalin (fig. 6).

The inferences derived from these results are the following. The amounts of mediator liberated on sympathetic nerve stimulation are directly proportional to the frequency of stimulation (p. 214). Sympathin differs from adrenine (p. 215). The data can be explained by assuming sympathin to be a compound of the mediator with the receptive substance (p. 215).

A mathematical analysis of the results is presented (p. 218).

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THE EFFECT OF VAGOTOMY ON GASTRIC EMPTYING TIME[1]

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Since the work of McCrea, McSwiney and Stopford (1927) and Hughson (1927) it has been generally believed that double vagotomy has little permanent effect on gastric motility. After an initial period of inhibitional paresis, a decrease in the initial emptying time was the only pronounced and constant finding reported by these authors. The growing evidence that the action of both vagi and sympathetics depends on the state of muscular tonus in the gastric muscle has seemed to lend additional support to their views. The opposite experiences of earlier workers were believed to result from inadequate methods, too brief a duration of the experiments and the intervention of shock.

The literature to 1931 has been reviewed by McSwiney (1931). Since then there have been few references to the subject. Cannon (1933) evidently still subscribes to his early views and believes that the initial inhibition of McCrea, McSwiney and Stopford is really the loss of gastric tonus for which the vagus is normally responsible. According to Cannon the vagotomized gastric muscle gradually develops intrinsic tonus. He therefore agrees with the previous authors that the final defects are slight. Both Quigley (1930) and Fetter, Barron and Carlson (1932) noted delayed emptying times in vagotomized dogs which were however devoted to other purposes.

Our purpose in the work here reported has been not only to study the effects of vagotomy itself, but to find if the discordant results of previous investigators might not depend on variations in the type and consistency of the test meals.

METHODS. Under ether-morphine anesthesia, strict asepsis and positive ventilation, the vagi were reached by resection of the seventh rib. A centimeter or more was removed from the vagal trunks which at this level usually consist of two or three strands on the left side and one on the right. With the exception of the gastro-intestinal disturbances to be described, the animals have recovered almost immediately without any pneumonia or pleurisy.

[1] This work has been made possible in part by a grant from the Wisconsin Alumni Research Fund.

For our purpose the initial and final emptying times of the stomach have been taken as an index of its motility. The determinations were made by the use of the fluoroscope. The animals were placed on their side without restraint and they quickly became so accustomed to the examinations that psychic and reflex disturbances were undoubtedly at a minimum if not altogether absent.

Barium meals of three kinds have been used. The first consisted of Champion dog biscuit thoroughly soaked in milk. Twelve and one-half grams of biscuit plus 4.1 grams of barium sulphate were given per kilo body weight. The second consisted of 12.5 cc. of whole milk plus 4.1 grams of barium sulphate per kilo. The third was the mixture used by McCrea, McSwiney and Stopford which contained 200 cc. of milk, 60 grams of bread, 7 grams of barium sulphate and one gram of sugar for each dog irrespective of weight.

It will be noted that the quantity of food was adapted to the weight of the animal. Unless the animals selected are of uniform size this is believed to be necessary for comparable experiments.

One of the greatest difficulties met in our experiments has been to make sure that the stomach was entirely empty when the test meal was given. Since any moderate degree of fasting did not accomplish this purpose we resorted to the subcutaneous injection of 1 mgm. apomorphine. The effects of the resulting emesis were short lasting and within half an hour the animals were usually eager for food.

Normal emptying time. The final emptying time of normal dogs according to the x-ray method has been determined by a number of workers. McCrea, McSwiney and Stopford report 5 to 7 hours for three animals; Ivy (1930) gives an average of 4 hours and 50 minutes for seven dogs; and Van Liere and Crisler (1933) give an average of 6 hours and 36 minutes for twenty-five dogs. Eight hours is approximately as long as any one has reported for any one animal. Variations in such studies must be rather large due to differences in test meals and peculiarities of the animals which cannot be controlled. It is safe to assume that any total period of emptying much longer than 8 hours indicates abnormal motility of the stomach. McCrea and his co-workers have also made use of the initial emptying time which they found to be from 5 to 16 minutes.

In our series of ten animals we have determined the initial and final emptying times on seven dogs for milk and barium and on three for biscuit and barium. In addition to these, which were actually used in experiments, we have other determinations making a total of twenty-two normals for milk and ten for biscuit. The average initial emptying time of all animals for milk is 11 minutes with a variation of 2 to 30 minutes. For biscuits the average is 17 minutes with a variation of 7 to 48 minutes. The final emptying times have averages 4 hours 27 minutes for milk with

a variation from 2 hours 36 minutes to 7 hours 50 minutes. For biscuits the final average emptying time is 5 hours 45 minutes with a variation from 2 hours 42 minutes to 9 hours 16 minutes. It will be noted that the initial emptying time for milk is slightly but definitely shorter than for biscuits.

Immediate effects of vagotomy. After the anesthesia has disappeared the dog appears perfectly normal until he attempts to eat. During this procedure deglutition is much in evidence. The dog eats a small portion of his meal and then ceases eating and makes several swallowing movements as though the bolus had lodged in his pharynx. He may then continue to eat or he may quietly regurgitate some of his food which he immediately eats again. Even when not eating there is some regurgitation of mucus and saliva. The dog gradually learns to eat his food slowly with frequent interruptions, and rather quickly the signs mentioned largely disappear. At no time do the symptoms seem to involve anything more than the mildest discomfort. The fluoroscope shows that the difficulties have been due to paralysis of the lower esophagus. A gradual improvement in this condition has been noted by other workers. The dogs at first lose some weight but this is gradually regained. At first the appetite is definitely abnormal. The dog is always hungry and stuffs his stomach with food, feces, shavings and anything else available. This condition is not believed to result from any sensation of hunger, since hunger waves only appear in the vagotomized animal when the stomach is empty, but it is due to a loss of the feeling of satiety which depends upon the integrity of the vagus. Once adjusted vagotomized dogs may live indefinitely in excellent condition.

Final emptying time after vagotomy. For convenience we have tabulated the emptying times at periods of two weeks, one month, three months, four months and five months. The first period has been chosen because it is within the time in which previous workers have reported the greatest changes.

The most important facts shown by the data in tables 1 and 2 are the immediate lengthening of the final emptying time and the persistence of this condition for at least as long as five months. Within two weeks post-vagotomy the average final emptying time was four to five times normal for both biscuit and milk. At the end of five months when the dogs were all in the best of health and at normal weight it still averaged about three times the normal length. The figures secured at this latter time may be accepted with confidence. They were duplicated on each animal and their general uniformity is the result of the uniformly excellent physical condition of the subjects. Since the changes from the fourth to the fifth month are slight there is little doubt that the longer final emptying after vagotomy is a permanent phenomenon.

The observations made within the two weeks period do not differ ma-

terially from those made at the end of a month. This is evidence that if there is any shock from a simple thoracic vagotomy such as we have carried out, it is at least short lasting. The animals show no signs of any general depression during the early period and we agree with Cannon that this early condition is the result of the vagotomy rather than some form of shock.

Although a delayed final emptying time is permanent, at least two animals, nos. 2 and 6, show a considerable improvement at the end of five months over their previous record. Others also show some improvement, for the average length of the final emptying is higher at the end of one month, after which there is a gradual shortening. Averages in a series as small as these are apt to be misleading, but the close agreement in the determinations at the fourth and fifth month periods would seem to show that improvement for such diets as biscuit and milk with barium has come to an end.

Effects of variations in consistency of test meals. It is possible that discrepancies in the literature regarding final emptying times after vagotomy may be due at least in part to the kind of food used. With this thought in mind we have tried out the effect of varying the consistency of the test meal. For this purpose we have used milk alone, and solutions of sucrose and peptone; gelatin, and a mixture of bread, milk and sucrose. The emptying times for the first mentioned substances were determined by aspiration and forced emesis; for the latter two by the use of barium and the fluoroscope. The results of these studies may be seen in table 3. The time after vagotomy at which the experiments were carried out was variable but those on milk fell within the two months period and the remainder from two to three months.

The most important thing shown by the data is that foods in fluid form are entirely discharged from the stomach of vagotomized dogs in normal or less than normal time. The time for milk alone averaged 4 hours and 58 minutes although the quantity of fluid in the test meal had been increased nearly four times.

The specified peptone and sucrose solution was chosen because it was non-coagulable and equal to milk in its concentration of total solids. Four dogs tested with this fluid showed emptying times well below the normal for biscuit or milk, one emptying a meal of 116 cc. in the remarkably short time of 9 minutes. The accuracy of the aspiration method in determining gastric emptying was checked in each case by the ability to recover a measured amount of water.

To visualize these results for fluids, gelatin which had a total solid concentration equal to that of milk and which would not gel at body temperature, was used with barium.

With this solution only one of five dogs showed a final emptying time longer than normal and this exception was well below the time for biscuit.

The mixture of bread, milk, sucrose and barium was used not only to duplicate the experiments of McCrea, McSwiney and Stopford but to furnish a test meal low in indigestible solid content, and intermediate in total solids between biscuit and barium and milk and barium. Four of these animals showed total emptying times shorter than they had for biscuit. One was approximately the same and one was longer.

The results of all the determinations in tables 1, 2 and 3 indicate that the emptying time in vagotomized dogs depends on fluidity and the amount of indigestible solid in the meal. For example peptone and sucrose, entirely fluid, with no indigestible barium gives the shortest emptying time. Milk alone, although the quantity used was large, gave an average second in the series. The delay here is evidently due to the formation of solid curds which must await digestion. Gelatin and barium fall a little behind milk. The figures are, however, strikingly shorter than for milk and barium, quite likely because of the fact that the barium is much better held in suspension by the gelatin. The mixture of bread, milk, sucrose and a small amount of barium is next in order of emptying. The two test meals taking the longest time for final emptying are biscuit and barium and milk and barium. The reason for this we believe is the large amount of indigestible barium used in each meal.

On the basis of the above mentioned experiments one would expect a much shorter emptying time with the more fluid meal of milk and barium than with biscuit and barium. However, a comparison of the results in tables 1 and 2 shows that although the time for milk is shorter, it does not have the marked advantage expected. The explanation for this discrepancy probably lies in the coagulation of the milk and sedimentation of the barium sulfate. Reference to the curves in figure 1 shows that immediately after feeding the rate of gastric discharge was actually much greater in the case of milk than biscuit. In the period of rapid emptying, undoubtedly the stomach discharged the fluid chyme and then later slowly discharged the barium and coagulated casein. In at least one dog this conclusion was substantiated by the large solid curds which were vomited after apomorphine.

Initial emptying time after vagotomy. The immediate effect of vagotomy on initial emptying time was variable in our experiments. About half showed a decrease while the others remained normal or increased. The initial emptying time after vagotomy, as well as before, also depends in large part on the fluidity of the meal. At any given post-operative period the average time for milk and barium is always less than for biscuit and barium. Gelatin and barium in five different animals began to pass out of the stomach without any delay whatever.

McCrea and his collaborators believed that an earlier initial emptying of the stomach was the most important permanent feature of vagotomy.

TABLE 1

Gastric emptying after a meal of milk and barium

EMPTYING	DOG 1		DOG 2		DOG 3		DOG 4		DOG 5		DOG 6		DOG 7		DOG 8		DOG 9		DOG 10	
	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes
Normal																				
Initial...		9		8		5								14		5		9		3
Final....	4	2	4	8	2	54							5	54	3	35	5	54	4	47
Av. initial, 9.8 minutes Av. final, 24 hours 47 minutes																				
Within 2 weeks post-vagotomy																				
Initial...		14		12		15		4		25		6						1		1.5
Final....	25	17	25	0	24	0	18	0	21	0	21	0					50	19	13	44
Av. initial, 41 minutes Av. final, 20 hours 6 minutes																				
Approximately 1 month post-vagotomy																				
Initial...		61		45		5		2		32		85				62				
Final....	18	30	18	30	18	30	11	12	21	0	24	0			29	0				
Av. initial, 15 minutes Av. final, 20 hours 13 minutes																				
Approximately 3 months post-vagotomy																				
Initial...		46		16		1		2		1		22								
Final....	34	30	2	0	7	12	12	0	15	0	15	23			32	30				
Av. initial, 4.4 minutes Av. final, 15 hours 5 minutes																				
Approximately 4 months post-vagotomy																				
Initial...				8		4		1		8		1								
Final....			24	21	12	1	12	34	15	21	11	7								
Av. initial, 2.6 minutes Av. final, 13 hours 17 minutes																				
Approximately 5 months post-vagotomy																				
Initial...				3		1		3		2		3.5		3						
Final ...			12	50	13	10	11	50	16	25	12	53	12	38						

Average initial for 10 normal dogs = 11 minutes.
Average final for 10 normal dogs = 4 hours 27 minutes.

TABLE 2

Gastric emptying after a meal of biscuit and barium

EMPTYING	DOG 1		DOG 2		DOG 3		DOG 4		DOG 5		DOG 6		DOG 7		DOG 8		DOG 9		DOG 10	
	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes
Normal																				
Initial....		12		11		32														
Final.....	3	33	3	28	5	26														
Av. initial 27 minutes Av. final, 21 hours 44 minutes																				
Within 2 weeks post-vagotomy																				
Initial....		51		63		37		16		5		11						34		3
Final.....	26	26	42	0	16	17	11	21	9	19	21	0					33	36	13	58
Av. initial 49 minutes Av. final, 28 hours 6 minutes																				
Approximately 1 month post-vagotomy																				
Initial....		81		56		12		35		27		81								
Final.....	39	0	39	0	25	0	14	0	26	39	25	0								
Av. initial 37 minutes Av. final, 25 hours 23 minutes																				
Approximately 3 months post-vagotomy																				
Initial....		82		37		13		7.5		26		55								
Final.....	27	0	41	0	17	33	11	28	23	14	14	30			43	0				
Av. initial 27 minutes Av. final, 19 hours 27 minutes																				
Approximately 4 months post-vagotomy																				
Initial....				55		4		8		13		58								
Final.....			29	5	11	45	12	39	24	22	19	27								
Av. initial 25 minutes Av. final, 19 hours 40 minutes																				
Approximately 5 months post-vagotomy																				
Initial....				19		2		58		22		30		20						
Final.....			19	4	19	8	16	43	26	8	15	55	21	5						

Average initial emptying time for 22 normals = 17 minutes.
Average final emptying time for 22 normals = 5 hours 45 minutes.

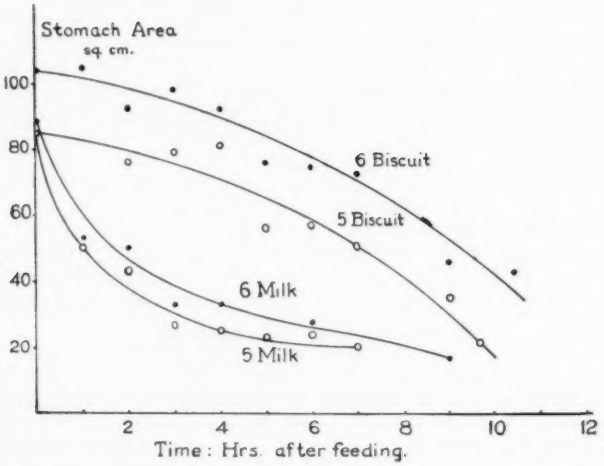

Fig. 1. Rate of discharge in dogs 5 and 6 for biscuit and barium and milk and barium as determined by the stomach area on the fluoroscopic screen.

TABLE 3

Final emptying time with fluids

DOG NO.	TYPE OF FLUID											
	Milk alone			6.5 per cent peptone and 6.6 per cent sucrose			Mixture of 12 per cent gelatin, 1.5 per cent NaCl and $BaSO_4$			Mixture of 60 gms. bread, 200 cc. milk, 1 gm. sucrose and 7 gms. $BaSO_4$		
	Time		Quantity	Time		Quantity	Time		Quantity	Time		Quantity
	Hours	Minutes		Hours	Minutes		Hours	Minutes		Hours	Minutes	
			cc.			cc.			cc.			cc.
1	6	0	627	2	45	558				24	20	268
2	5	30	509	4	45	614	11	0	220	19	0	268
3	3	10	468				3	18	160	11	15	268
4	5	0	350				3	16	120	20	43	268
5	5	0	475				5	18	167	24	0	268
6	5	0	560				4	10	220	13	30	268
9	5	0	600	2	5	200						
10	5	0	480		9	116						

Data in this table indicate the time necessary for a vagotomized stomach to discharge the given quantity of fluid.

Since initial emptying times are actually short under all conditions, individual variations often make it difficult to decide whether vagotomy has really reduced this interval. Our data however for milk and barium justify the statement that vagotomy has really reduced this interval. All five of the animals most intensively studied showed a definitely decreased initial emptying time for milk and barium at the end of the fourth month and this was maintained for the fifth month as well. The solid part of this diet was retained far beyond the normal time although some of the fluid part entered the intestine almost as soon as the animal could be placed on the fluoroscopic table. The diet used by McCrea and his collaborators being rather high in fluid content was particularly suitable in bringing out this reduced initial emptying time.

Although at the end of two weeks post-vagotomy four animals showed a shortened initial emptying time with biscuit and barium this was not maintained and at the end of five months only one of six dogs had an interval below normal.

To all appearances it would seem impossible for a vagotomized animal that retained food in its stomach for twenty to thirty hours to deliver food rapidly enough to the small intestine to regain weight and maintain a normal nutrition. Some light has been thrown on this situation by feeding our animals the usual test meal of biscuit every eight hours and measuring the stomach areas as projected on the fluoroscopic screen. Some six animals have been studied in this way. The results have been reasonably uniform and a typical experiment is shown in figure 2. It must be recognized that silhouette areas of the stomach cannot be quantitatively related to the mass of food ingested with any great degree of exactness because of the irregularity of outline and the fact that the food may be spread out in layers of different depths. The curves, however, show that by eight hours after each feeding the stomach volume had been reduced to a more or less average pre-feeding level, represented by an area of approximately 80 square centimeters. In other words, if this dog's stomach was kept in a certain degree of distention, he was able to discharge in eight hours a meal which otherwise required twenty-five hours. This he continued to do for four days with a total of twelve feedings.

These results are interesting in two connections. In the first place they explain how an animal with food ad libitum may keep up an approximately normal delivery of food to the intestines after vagotomy and thus regain his normal weight and maintain his nutritive processes. That the dogs do take advantage of this means of discharging their food from the stomach is indicated by forced emesis which at all times has shown a considerable volume of gastric contents. Secondly, these experiments also have a bearing on gastric tonus. Our observations have indicated that the

tonicity of the gastric muscles depends in a marked degree on vagal innervation. Immediately after vagotomy, fluoroscopic examination shows a dilated stomach full of air. If fluid test meals are given the stomach becomes more pendulous and on shaking the animal the fluid may be seen to splash around freely. Fluid may leave the stomach almost at once. If milk soaked biscuits are given the outline of the stomach is ragged and the solids leave slowly. In the normal animal the stomach outline is smooth with both these diets, air is absent and the contents cannot be made to splash. The explanation can only be that the vagotomized

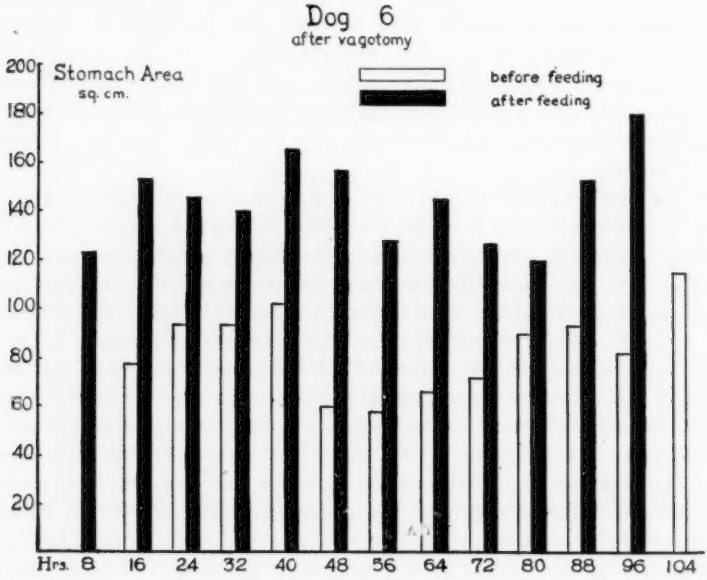

Fig. 2. Stomach areas on the fluoroscopic screen before and after eight hour feedings.

animal has almost entirely lost its gastric tonus. With frequent feedings a large enough residuum is retained so that the stomach is kept in a more or less stretched condition. This, of course, favors the development of an inherent tonus and on this tonic level excess contents are delivered in a fairly normal fashion.

The permanent loss of gastric tonus after vagotomy is sufficient to explain the decreased initial emptying time for fluids as well as the increased final emptying time for solids. A certain amount of fluid may pass out with little more than gravity pressure behind it. Solids, however, need the motor power of the gastric muscle in order to be moved along.

Any decrease in the final emptying time would be an indication of recovery to the same degree in gastric tonus. Our experiments are in harmony with the general idea of stomach emptying already expressed by Thomas (1931).

SUMMARY

Thoracic vagotomy in the dog has lengthened the final emptying time of the stomach from three to four times for diets of milk or dog biscuit with barium. This change persists for at least five months and it is believed to be permanent.

After the immediate variable effects the initial emptying time for milk and barium is shortened by vagotomy. This shortening could not be demonstrated for a more solid diet of dog biscuit and barium.

The final emptying time of fluid diets which could form no curds or precipitates, such as peptone and sucrose or gelatin, remained at normal figures after vagotomy.

The amount of fluid in the test diet is believed to account for most of the discrepancies in the literature on gastric emptying times after vagotomy.

If the stomach is kept permanently distended the addition of further food allows emptying to proceed at a fairly normal rate.

All the evidence points to the vagus as a nerve necessary for the existence of normal gastric tonus and that emptying depends in some way on the degree of this tonus.

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THE EFFECT OF 2-4-DINITROPHENOL ON THE OXYGEN UPTAKE OF KIDNEY AND LIVER TISSUE OF THE RAT[1]

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When 2-4-dinitrophenol is introduced into the animal body, an increase in metabolism takes place. This increased metabolic rate has been shown to be independent of the nervous and endocrine systems (1), for isolated strips of muscle and sections of other tissues show an increased metabolism in the presence of dinitrophenol. In the treatment of conditions in which it seems desirable to increase the metabolism, Cutting, Mehrtens and Tainter (2), Tainter, Stockton and Cutting (3), and Cutting and Tainter (4) report favorable results, while Anderson, Reed and Emerson (5), and Geiger (6) report unfavorably. The purpose of the present study was to determine the effect of widely varying concentrations of dinitrophenol upon the increase or decrease in the oxygen uptake of tissues.

EXPERIMENTAL PROCEDURE. Normal healthy rats three or four months old and weighing from 120 to 140 grams were selected. These animals, all in a good state of nutrition, were placed on an eighteen-hour fast, after which they were killed by stunning and decapitation. Immediately after death the liver and kidneys were removed and placed under the respiration medium. The livers were then sectioned, the sections placed in the Warburg respiration bottles, and the oxygen uptake measured over a period of two hours.

The measurements upon kidney tissue were made after the kidneys had been allowed to stand in the ice box for approximately six hours under the respiration medium. A few comparisons were made between the respiration of the kidney tissue immediately after death and after having stood for a period of six hours in the ice box. Very little, if any difference was noted.

M/30 NaH_2PO_4, adjusted to a pH of 7.4 with sodium hydroxide, and with 0.2 per cent glucose was used as the respiration medium (7). Inasmuch as this medium differs so greatly from normal blood serum, its use is open to severe criticism. For this reason, we made preliminary ob-

[1] A preliminary report of these studies was given before the Biochemical Section of the American Chemical Society at the 87th meeting at St. Petersburg, Fla., March 25, 1934.

servations for the purpose of determining to what extent and for what length of time the tissue was capable of taking up oxygen in this medium (see table 1).

TABLE 1

Average O_2 uptake of three determinations on each of 10 animals in M/30 NaH_2PO_4 and 0.2 per cent glucose. pH = 7.4

	LIVER				KIDNEY			
	30 min	60 min.	90 min	120 min	30 min	60 min	90 min	120 min
Total O_2 uptake cmm./ mgm. dry tissue.....	3.2	5.7	7.9	10.0	8.8	16.3	23.0	27.9
O_2 uptake in 30 min. periods.	3.2	2.5	2.2	2.1	8.8	7.5	6.7	4.9
O_2 uptake per hour.....	6.4	5.0	4.4	4.2	17.6	15.0	13.4	9.8

TABLE 2

O_2 uptake in 2–4-dinitrophenol in cubic millimeters per milligram dry tissue per hour

CONC. D.N.P. IN GRAMS	RAT LIVER				RAT KIDNEY			
	RO_2 in D.N.P.	RO_2 control	Per cent of variation	Average per cent variation	RO_2 in D.N.P.	RO_2 control	Per cent of variation	Average per cent variation
1– 100,000	5.4	6.8	−20	−20	15.1	25.3	−42	−42
1– 500,000	7.6	8.0	−5.0	−5.0	19.3	24.0	−19.5	−19.5
1– 1,000,000	5.0	5.3	−5.7	−5.7	13.7	17.1	−20	−20
1– 2,000,000	5.5	6.2	−11.3	−11.3	21.3	25.3	−15.8	−15.8
1– 5,000,000	5.2	5.7	−8.8		17.6	18.7	−5.8	−5.8
	5.3	5.7	−7.0	−7.9				
1–10,000,000	5.5	5.2	+5.8		19.3	21.3	−9.5	
	4.0	4.7	−15.0		21.1	20.7	+1.9	
	6.6	5.9	+11.9		17.8	15.4	+15.6	+2.7
	10.0	8.9	+12.4	+3.8				
1–20,000,000	9.9	6.3	+57		15.3	11.9	+28.5	
	6.4	5.8	+10.4		20.1	17.1	+17.5	
	4.0	3.8	+5.3		17.9	15.6	+14.8	+20.3
	5.5	5.5	0					
	7.1	6.5	+9.3	+18.4				
1–40,000,000	6.0	5.7	+5.3		16.1	17.7	−9.0	
	6.1	6.2	−1.6	+1.8	14.9	13.9	+7.2	
					16.7	16.8	−5.8	−2.3

Kisch (8) has shown that the addition of phosphate to Ringer's solution results in a steady and gradual decrease in respiration. We have observed that both kidney and liver sections will absorb oxygen normally for a limited length of time in the NaH_2PO_4 medium. However, there is a steady and gradual decrease in the oxygen uptake which may fall 30 per

cent in two hours in the case of liver tissue, and as low as 50 per cent in the case of kidney tissue. After a period of from four to six hours, the oxygen uptake usually falls to zero.

2-4-dinitrophenol was added to the buffer medium to give concentrations of the drug varying from 1–100,000 to 1–40,000,000. Four determinations of the effect of 2-4-dinitrophenol were made upon the liver and kidneys of each animal. Three control determinations in the phosphate-glucose medium alone were made simultaneously upon adjacent tissue sections to insure accurate comparisons and to eliminate errors due to a variable sensitivity of various animals to the drug. The average for the experimental and control values for each animal are shown in table 2.

DISCUSSION. The results obtained indicate that 2-4-dinitrophenol will increase the oxygen uptake of tissues in optimum concentrations of about 1–10,000,000 to 1–30,000,000. However, when the concentration is higher than 1–5,000,000 a decided decrease in oxygen uptake takes place showing that high concentrations of dinitrophenol in the tissues themselves will inhibit respiration rather than increase it. Oral, subcutaneous or intravenous administration of dinitrophenol in large doses result in an increased metabolism, as manifested by pyrexia and other symptoms (9). A possible explanation of the increase in the rate of metabolism under these conditions rather than a decrease, is that the tissues respond immediately to the drug as it is absorbed, and before it is absorbed in quantities large enough to cause a decrease. In some particular cases the response to the drug varied widely. In a concentration of 1–20,000,000 in the case of one particular animal, the metabolic rate was increased 57 per cent, while on another it had no effect at all. Clinically, this variation has been demonstrated by the fact that seven per cent of the patients to whom the drug was administered exhibited allergic symptoms (3). No explanation is offered for this variation except that some of the animals in the series were apparently more susceptible to the drug than others.

This optimum concentration of about 1–20,000,000 for the rat differs markedly from the optimum concentration for frog tissue (10) which has been reported as 0.5 mgm. per cent, or 1–200,000. This is not surprising, for other drugs vary greatly in optimum concentrations in their action on cold- and warm-blooded animals. A considerable variation may be noted, even in different animals of the same species, in the effect of dinitrophenol.

SUMMARY

2-4-dinitrophenol, added to a respiration medium of M/30 NaH_2PO_4 and 0.2 per cent glucose in Warburg vessels, increases the oxygen uptake of rat liver and kidney tissue when present in a concentration of about 1–20,000,000 but decreases it in a concentration higher than 1–5,000,000.

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THE NORMAL BEHAVIOR OF THE PULMONARY BLOOD VESSELS WITH OBSERVATIONS ON THE INTERMITTENCE OF THE FLOW OF BLOOD IN THE ARTERIOLES AND CAPILLARIES[1, 2]

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In the work described in this paper, a method has been developed for direct microscopic observation of the smaller blood vessels of the mammalian lung in the unopened thorax. The behavior of these vessels and the flow of blood through them have been studied under a variety of conditions. We were particularly interested in their normal behavior, in the question of intermittence of the flow of blood in the arterioles and capillaries and the factors controlling it, and in the number of capillaries open at a given time and the variations in the circulation within them. Observations upon the actions of various drugs on the pulmonary vessels are also presented.

When this work was begun in 1924, Krogh (1919 and 1922) and Richards and Schmidt (1924) had reported the results of their brilliant work in which the Malpighian method of direct microscopic observation was used. The work of these investigators suggested the possibility of the re-adaptation and modification of the Malpighian method for use in studying pulmonary blood vessels in the cat.

Microscopic observations on the pulmonary circulation of the frog were first made and reported by Malpighi (1687). Stephen Hales (1733) and Tiemann and F. Roeder (1932) have also observed the flow of blood in the

[1] The expenses of this investigation have been defrayed in part by a grant from the DeLamar Mobile Research Fund of the Harvard University Medical School and in part by the P. W. Harvey Research Fund in the Department of Medicine of Lakeside Hospital.

[2] Doctors Barr and German, while fourth year students in the Harvard Medical School, spent a year in my laboratory and took part in developing the method as well as in some of the earlier experiments. Doctors Ernstene and Bromer, and Miss Zschiesche took part in a majority of the observations upon the behavior of the vessels and the action of drugs upon them.

blood vessels of the frog's lung. No intermittence in the circulation was noted.

Cohnheim and Litten (1875) injected dye into the blood streams of rabbits and found that it was unevenly distributed in the lungs while evenly distributed in the other organs. This work, which is supported by that of Toyama (1925), furnishes the first evidence of an intermittent flow of blood in the pulmonary vessels.

Hall (1925) transilluminated the lung in cats and rabbits and observed the pulmonary circulation by opening the chest and drawing out and fixing a quiet lobe of the lung with small clamps. He described the blood flow in the arterioles, capillaries, and veins and, in the larger arterioles and venules in which the walls were thin enough to see the cellular elements of the blood, he observed a pulsatile flow. In the smaller arterioles and capillaries, the flow was steady. He reported no intermittence of arteriolar or capillary circulation. Following epinephrine he noted a reversal of direction of circulation and in some instances a constriction of arterioles.

Olkon and Joannides (1930) described the capillaries of the alveoli in the lung of a living dog and observed changes in blood flow with change in intrapulmonary pressure.

MacGregor (1933) modified Hall's method to study the pulmonary vessels in the cat and in the isolated, perfused lung. He reported alteration in the rate and a reversal of flow but no spontaneous intermittence of arteriolar or capillary blood flow. It is interesting that the reactions he observed in local vessels did not always agree with the general reaction of the vessels of the entire lungs as measured by the inflow and outflow.

Wiggers (1921) and Daly (1933) have published excellent reviews of the literature concerning the pulmonary circulation.

METHOD. In our experiments, the cat was used because its parietal pleura proved to be best suited to the method employed, the pleura of the dog being too thick and that of the rabbit too fragile.[3] Amytal in a dosage ranging from 50 to 100 mgm. per kgm. was given intraperitoneally in sufficient amount to secure light but complete anesthesia. The normal body temperature, as determined by a rectal thermometer, was maintained by a heating pad.

The lung was brought into view by dissecting away the muscle in the mid-axillary line between the eighth and ninth ribs until only the parietal

Wearn, Barr, and German (1926); Wearn, Ernstene, Barr, and German (1927); and Wearn, Ernstene, and Bromer (1928) reported some of the experiments, the results of which are given in full in this paper.

[3] The lung of the frog lends itself beautifully to microscopic study but repeated observations carried on for hours at a time revealed a steady blood flow through the pulmonary vessels. These results confirm those of Tiemann and F. Roeder. These observations were made while I was in the laboratory of Prof. A. N. Richards in the University of Pennsylvania in 1921–23. J. T. W.

pleura remained as a clear, transparent, intact membrane. The diameter of this pleural window usually ranged from 0.6 to 1.2 cm. Through such a window the lower edge of the lower lobe of the lung was usually revealed. The pleura was kept moist by frequent applications of normal salt solution at body temperature. Through a mid-line abdominal incision another window was made in the diaphragm by dissecting the muscle from the abdominal surface of the diaphragm until the parietal pleura was exposed. This window was placed immediately opposite the one in the chest wall so that the tip of the lung lay between them. A beam of light from an arc lamp was passed through a cooling chamber and a quartz rod and thrown through the diaphragmatic window in such manner as to transilluminate the tip of the lung and make possible observations of the pulmonary vessels with a microscope at the window in the chest wall (fig. 1). All observations were made with a Spencer binocular bi-objective microscope at a magnification of ninety or one hundred and seventeen diameters. The blood pressure was recorded by means of a cannula in the carotid artery

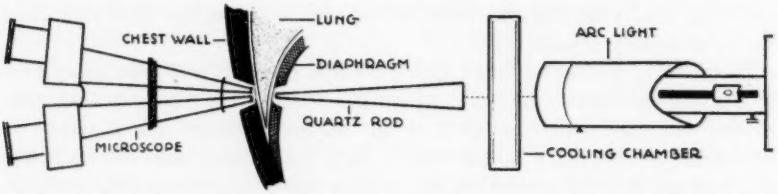

Fig. 1. Shows the detail of the position of the lower border of the lung in relation to the microscope and lighting system.

and the femoral vein was exposed for the injection of drugs and other substances.

METHOD OF OBSERVATION. Our observations were carried out in two groups of experiments. In the first, the cats were breathing, while in the second group, the lungs were immobilized by the use of curare.

In the first group of experiments the difficulty of observing the moving lung grew less as our eyes became accustomed to the motion. Morphine was used to slow the animals respiratory rate, but even with the slow rate we did not feel secure in detecting the finer capillary changes and for this reason the lungs were immobilized in the second group of experiments.

Curare[4] was injected into the femoral vein in sufficient dosage (usually 1.0 to 1.5 cc. of a 1.0 per cent solution) to abolish respiratory movements. A small catheter was introduced into the trachea for inflation of the lungs

[4] We are indebted to Dr. C. K. Drinker for the high grade curare used in our earlier experiments. We later obtained a high grade curare from Peru. The condition of the animals remained satisfactory for several hours after the use of each.

which amounted to a modification of Meltzer's method (1909). Air was passed through a water bottle and the pressure in the trachea was so regulated as to keep the tip of the lower lobe of the lung between the pleural windows. At short intervals the chest was compressed several times by hand to insure proper ventilation. This procedure did not interfere with the return to view of the air sacs under observation. By this method we secured a still lung which greatly facilitated our observations and gave us confidence in their accuracy.

The fact that the behavior of the vessels was the same in the moving and still lungs excludes the possibility of the vascular reactions being caused by the respiratory movements. Likewise the maintenance of good ven-

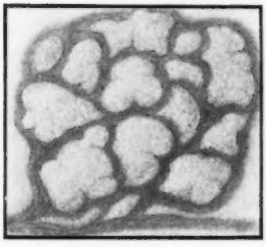

Fig. 2

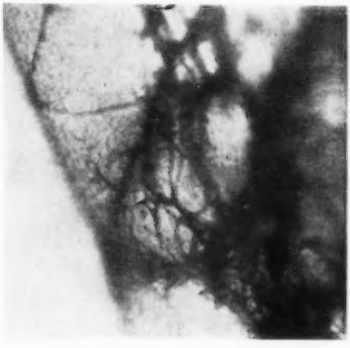

Fig. 3

Fig. 2. An artist's sketch of air sacs and cells showing their relation to the blood vessels surrounding them. No vessels are shown on the surfaces of the air sacs.

Fig. 3. Photograph of the lower edge of the lung. It shows the size and shape of the air sacs and cells. The heavy, dark lines are blood vessels and lymphatic channels. No vessels are visible on the surfaces of the air sacs. Approximate magnification 100 diameters.

tilation eliminates asphyxia as a causative factor in the reactions of the vessels described in this paper.

The following observations were made in both the moving and immobilized lungs. Observations which were not common to both groups of experiments will be so indicated.

Air sacs and blood vessels. The vessels selected for study lay on or immediately below the lung surface nearest the microscope. The air sacs and air cells with their borders sharply outlined by small blood vessels and dark pigmented lines were easily identified by their glistening, pinkish, semi-transparent walls (figs. 2 and 3).

The walls of all the smaller blood vessels were for the most part trans-

parent and therefore not visible, but the thickness of the wall of an arteriole could be judged with a fair degree of accuracy by the width of the transparent area lying between the line made by its outer wall against the adjoining structure and that formed by the blood column within the vessel. The walls of the larger arterioles were opaque.

Arterioles. Our observations were usually made upon arterioles of approximately one hundred and ten microns or less in diameter, inasmuch as the majority of the vessels of this size permitted a clear view of the blood cells within them. Gross changes were also recorded in larger vessels.

The flow of blood in the arterioles was sometimes pulsatile, more often steady and non-pulsatile but frequently changed from the steady to the pulsatile flow or vice versa. In a single microscopic field we often saw a rapid, steady flow in one arteriole, a sluggish steady stream in a second, and a slow pulsatile flow in a third. Indeed, a different type of flow may occur in two arteriolar branches of one parent arteriole. Moreover, in the smaller arterioles a variation in the cell volume of the circulating blood has been noted when the blood flow was sufficiently slow to permit such observations. The cells may be closely packed or they may pass through the arteriole in single file or two or more abreast with irregular clear spaces between them. Such spaces we interpret as the transparent circulating plasma. The moving blood column within an arteriole may stop suddenly or gradually, become pulsatile without moving forward, and then resume flow. As frequently observed, the blood flow in an arteriole and its capillaries may come to a stop and the cells may be expelled peripherally to leave the arteriole and capillaries invisible, or less commonly with the cessation of blood flow, the column may remain motionless in the arteriole.

Contractility of arterioles and intermittence of blood flow. In addition to the disappearance of arterioles, we have observed their spontaneous appearance with active circulation in a field where they had not been visible previously. Also in one instance pressure applied to the abdominal aorta resulted in the appearance of blood flow in an arteriole and several capillaries arising from it, which had previously been closed and invisible. After the pressure on the aorta was discontinued, blood flow ceased in the arteriole and the vessel began to constrict at a point about midway in its course across the surface of the air sac. The constriction divided the blood into two columns and the vessel emptied itself by expelling the columns in opposite directions until each portion disappeared from its side of the microscopic field.

In another experiment an arteriole resembling a capillary in that it permitted red blood cells to pass through it in single file, dilated sufficiently after an injection of epinephrine to allow corpuscles to pass through it eight or more abreast. (See sketch in fig. 10.)

In one arteriole—a branch of a larger arteriole in which there was a steady flow—a stationary blood column was observed which ended abruptly about one-third of the way across the surface of an air sac which it traversed. Beyond the point where the blood column ended, the vessel was invisible. Immediately after a small dose of epinephrine was injected, the blood column forced the vessel open and established a steady flow through it (see fig. 4, air sac *H*).

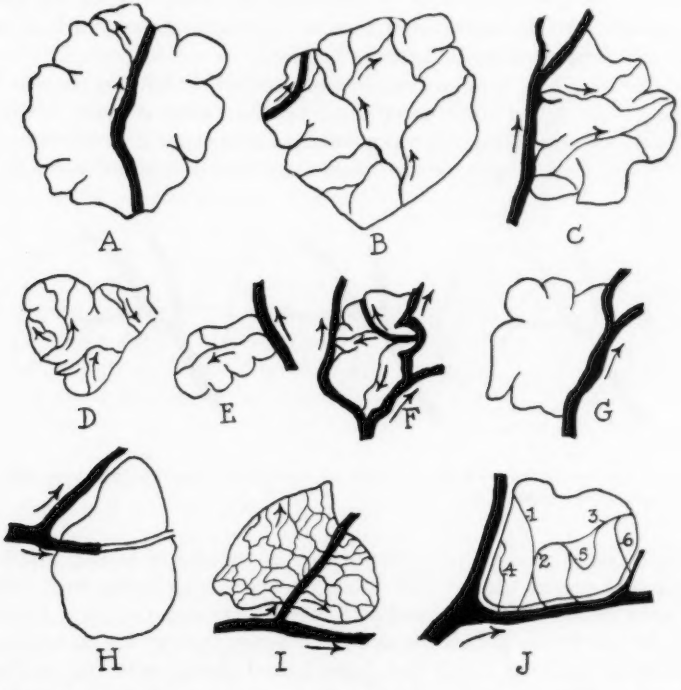

Fig. 4. Sketches of various types of circulation on air sacs. The broad dark lines represent arterioles and the finer lines capillaries (see text for special references).

In figure 4, air sacs *A*, *B*, and *F* are shown with an arteriole crossing their surfaces. When observations were first begun, these vessels were showing active flow but a few minutes later the arterioles closed and disappeared from view.

Another phenomenon observed frequently in the pulmonary arterioles is the spontaneous reversal of the direction of the circulation. In figure 4 the air sac *F* has arterioles lying on either side of it (the heavy black lines) with arrows indicating the direction of the blood flow at the beginning of

the observation. Suddenly the velocity of the blood flow decreased, the flow became pulsatile, stopped, and then began in the opposite direction. The direction of flow in the arteriole crossing the air sac did not change. After the reversal is once established the original velocity of flow may be resumed, and intermittence in the flow of the capillaries may occur. At times the blood flows in the reversed direction only momentarily, but in some experiments it has continued for several minutes. These changes are similar to those described by Hall and MacGregor after the use of epinephrine. We have produced them with epinephrine and various other drugs and these results will be dsecribed later.

Capillaries. The capillary walls were invisible, but the course and calibre of the vessel were determined by the blood column within it. With the lung immobilized, it was possible to make accurate sketches of the arterioles and capillaries on the visible surfaces of the air sacs (fig. 4).

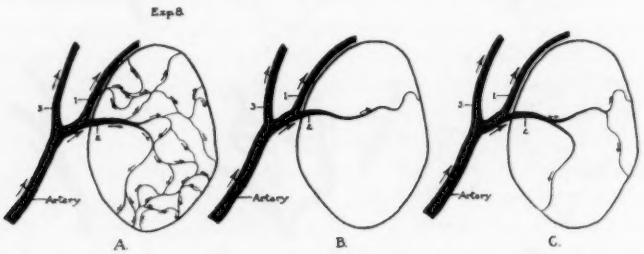

Fig. 5. Shows sketches of air sac and its vessels as observed at three different stages during experiment 8.

Under the conditions of our experiments, the number of active[5] capillaries per air sac[6] varied widely. At times no active capillaries were visible, and again the air sac was covered with them. The general range, however, was from two to ten per air sac and the average number for each group of experiments (moving lungs and immobilized lungs) was six capillaries per air sac. When the number of capillaries was more than ten per air sac, branchings and anastomoses were so frequently encountered that accurate quantitation was no longer possible. Indeed, a single capillary on the surface of an air sac should be looked upon as one channel of a meshwork of vessels and not as an isolated conduit. In a few experiments when the circulation was failing, and on other occasions without obvious cause, we have seen what we consider to be the entire capillary bed of the air sac

[5] By "active capillary" is meant a capillary through whose lumen blood is circulating.

[6] When we speak of the number of capillaries per air sac, we mean the number of active capillaries on the visible surface of an air sac.

filled with blood. In these instances, the surface of the sac did not appear to have individual capillaries, but resembled very closely a fine meshed netting made up of channels filled with blood (fig. 6-B). We have never been able to produce experimentally the state in which all the lung capillaries would open. As a general rule, when the heart began to fail, it was more common to observe a closure of all the lung capillaries.

Variation in capillary circulation. At first, the capillaries were extremely difficult to see because of their invisible walls. They appeared as processions of red blood corpuscles, usually in single file, but frequently two abreast, moving with such rapidity at times that they resembled blurred red lines. Again, the stream was sluggish, pulsatile, and moved very slowly, or, the most characteristic type of flow perhaps, was a single file of cells, almost invisible at first, but detected by their reflections of light as they either hurried or floated leisurely across the air sac in the plasma stream. Sometimes a single corpuscle entered and traversed the length of the capillary before the next cell appeared.

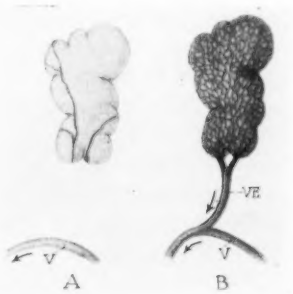

Fig. 6. Represents two sketches of the same air sac at different stages in experiment C-2. In figure A a few active capillaries are shown. In figure B the entire capillary bed on the air sac shows active circulation. The venule (*Ve*) appeared when all the capillaries opened. It was not visible when sketch A was made. Magnification approximately 117 diameters.

The final differentiation between an arteriole and a capillary was determined by the width of the blood column which its lumen would accommodate. A sharp classification must necessarily be an arbitrary one and in this paper all vessels whose lumens admitted three or less red blood cells abreast were called capillaries. Evidence of the difference in diameter of the various capillaries was furnished by the shape of the red corpuscles passing through them. When the vessel was constricted, the corpuscle seemed to be squeezed and in contact with the capillary wall, while other capillaries with the same parent arteriole allowed two corpuscles abreast without crowding.

Exact measurements of the length of the capillary channels have not been made but relative lengths of capillary channels have been determined in the following manner. Three sketches of the same air sac with a different circulation in each case were photographed and enlarged sufficiently to permit accurate measurement of the lines representing capillaries (fig. 5). Lead fuse wire was bent, superimposed over each capillary, straightened, and then measured. By this method, the length of the

capillary channels in figure 5-A was found to be approximately 3.6 times those in figure 5-C and 9.2 times those in figure 5-B.

In another experiment we were able to sketch an air sac which showed a few active capillaries in the beginning and which later showed what we believed to be its entire capillary network. Figure 6-A represents the air sac as it appeared at the beginning of the experiment. We were unable to trace the flow in the capillaries beyond the edge of the air sac, and venule (*Ve*) was not visible. At a later stage in the experiment, the entire capillary network on the air sac showed active flow and simultaneously the venule (*Ve*) opened to drain the flow into the venule (*V*) (fig. 6-B). Later all the capillaries closed. The arterioles supplying the capillaries were not visible at any time during the experiment. This and many similar observations furnish an excellent idea of the capillary reserve of the lungs. We do not claim that the sketches are exact, but we do feel that they are sufficiently accurate to justify our belief that the average number of active capillaries (six per air sac) represents a small fraction, perhaps one-tenth to one-fifteenth of the available capillaries per sac.

Spontaneous opening and closing of capillaries were observed in practically every one of our experiments. In order to study this behavior and the underlying causes of the intermittence of blood flow more intensively, we selected certain air sacs showing active arterioles and capillaries and recorded and timed the changes in them on the smoked drum. This was done with one individual at the microscope, another at the kymograph, and a third tending the animal. Changes in the circulation were frequently confirmed by a second observer at the microscope. When necessary, one took notes and kept time with a stop watch. A series of numbered keys, each connected with a signal magnet which recorded on a smoked drum, were arranged in a convenient place. Each capillary under observation was given a number and, when any change occurred in the capillary, the key of the corresponding number was so manipulated as to record the mark on the drum.

In the following experiment intermittence of blood flow in the capillaries was recorded. (Only excerpts from the protocol are quoted here.)

Experiment 2. April 4, 1927. Cat. Weight 2.01 kgm. Amytal 200 mgm. intraperitoneally. Curare 1 cc. 1.0 per cent solution I.V. Prepared as in method described above. After making several preliminary observations, an air sac was selected for study. (See fig. 4, air sac *J*.)

3:44 Capillaries 1 and 2 open; 1 shows rapid, steady flow; 2 slow, pulsatile flow.
3:46 Capillary 2 shows more rapid flow. Blood pressure 94.
3:48 Capillary 3 appears with rapid, continuous flow. Blood pressure 94.
3:49 Capillary 4 appears with rapid but intermittent flow. Blood pressure 94.
4:01 Respiratory movements beginning. Curare 0.25 cc. of 1.0 per cent solution given intravenously.
4:01:30 Flow in capillary 2 slow and pulsatile. Blood pressure 100.
4:03 Flow in capillary 2 becomes rapid and continuous. Others unchanged.

* * * *

4:17:36 Capillary 3 reappears and the flow in capillary 4 becomes very slow, only an occasional red corpuscle passing through. The flow in the arteriole from which this capillary arises remains unchanged. Blood pressure 80.

4:17:44 Capillary 4 disappears and almost immediately the flow in capillary 2 becomes slow and slightly pulsatile, while that in capillaries 1 and 3 remains unchanged.

4:27:40 Capillary 1 disappears as before. Blood pressure 81. Capillary 5 begins to show intermittent flow of cells which come in spurts of a dozen or so.

4:38:51 Capillary 5 reappears. Slight respiratory movements have begun. Blood pressure 74.

4:40:11 Capillary 4 appears with a rapid but intermittent flow. Apparently there is a rapid flow of plasma with single cells or groups passing through intermittently at irregular intervals. Flow in the parent arteriole unchanged. Blood pressure 75.

* * * * = Irrelevant parts of protocol omitted.

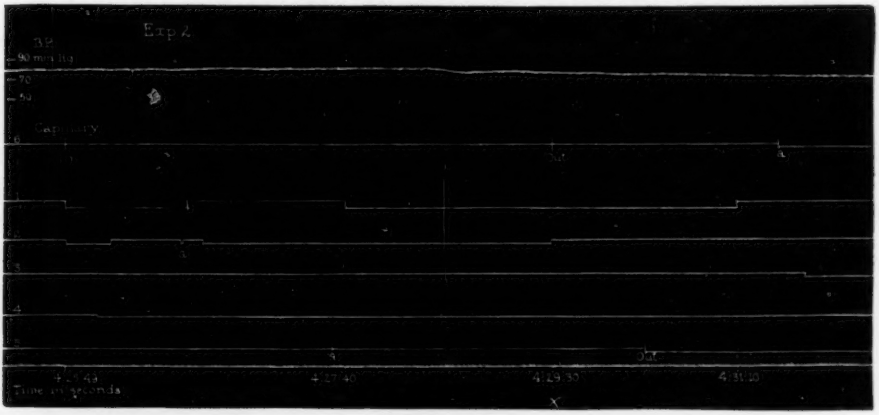

Fig. 7. Section of the kymographic record of experiment 2 showing blood pressure and time in seconds. The changes in the lines labelled *Capillary 1, 2, 3, 4, 5,* and *6* represent changes in the behavior of the capillaries under observation (see text). *In* = beginning of flow in the capillary. *Out* = cessation of flow. *A* = intermittent flow. Lever up = capillary showing active flow. Lever down = capillary closed.

A section of the original record of experiment 2 is shown in figure 7. The length of these records is such that publication of the entire records is impossible. They have been condensed and charted, therefore, as shown in figures 8, 9, 10, and 11.

This experiment shows quite clearly the intermittent flow in the capillaries of the lung as well as the variation of flow within them. Similar observations have been made repeatedly in our laboratory during all seasons of the year and at least seven observers have taken part. From these results and many other similar ones, we have concluded that inter-

mittence of blood flow in arterioles and capillaries of the lung represents the normal behavior of these vessels.

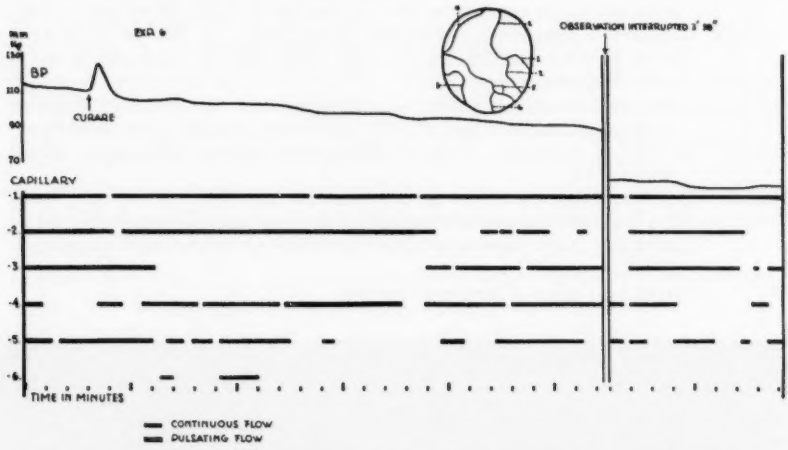

Fig. 8. Chart showing variations in the behavior of capillaries as observed on one air sac which is sketched in the chart. A dark line represents continuous flow, a cross line pulsating flow, and interruption of the line represents cessation of flow. This chart was constructed from data in the original kymographic record and our notes.

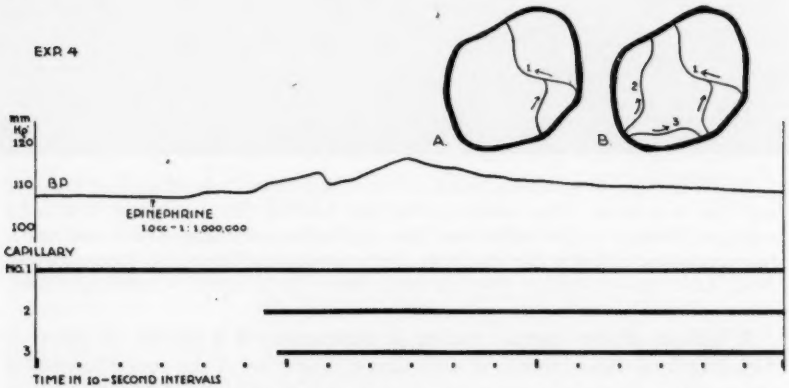

Fig. 9. Chart showing the effect of a small dose of epinephrine and sketches of the changes in capillary circulation before and after the drug.

It is generally agreed that the pressure curve in the pulmonary artery follows and runs approximately parallel to that in the aorta. In the protocol cited and in figures 7 and 8, in which the results of two experiments

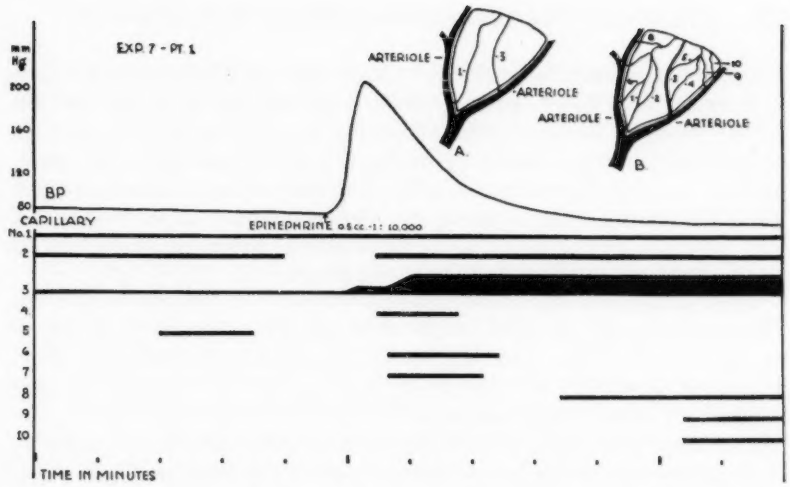

Fig. 10. (From expt. 7, part I, Jan. 12, 1928, prepared as described above.) Changes in circulation following 0.5 cc. of 1:10,000 solution epinephrine. The number of active capillaries increased and the vessel labelled *capillary 3* which had shown a circulation of red cells in single file dilated to become an arteriole with 8 or more cells abreast.

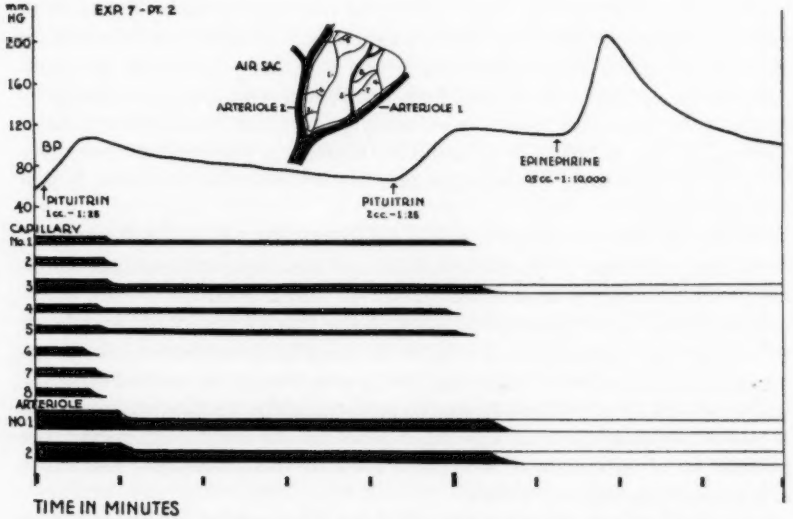

Fig. 11. Constriction and closure of arterioles and changes in the capillaries are shown after injection of pituitrin. The blood pressure rise following epinephrine failed to overcome the constriction produced by the pituitrin (see text). The broad lines represent increase in the velocity of blood flow in the capillaries and arterioles, the narrow lines slowing of flow, and interruption of lines cessation of flow. The parallel lines in capillary 3 and arterioles 1 and 2 represent stationary blood cells in these vessels.

are charted, evidence is produced to show that intermittence of the pulmonary capillary flow is certainly independent of changes in the systemic blood pressure. Moreover, the number of capillaries open and showing circulation is not determined by the level of blood pressure in any given animal. In many of the experiments where either high or low pressures existed at the beginning, only one or two capillaries per air sac were found and the equivalent of fifteen or thirty capillaries per air sac was found in the presence of both high and low blood pressure.

There is the possibility of independent changes in the pressure within the pulmonary circulation which might play some rôle in causing the intermittence in the blood flow within the arterioles and capillaries. It is not likely, however, that changes of great magnitude occur in the pulmonary pressure with the animal under such constant conditions.

Another possible explanation is suggested by these observations; namely, that the contractility shown by the arterioles in the lungs may play some part in regulating the pressure in the pulmonary circuit. What governs the contractility is unknown, but in many of our experiments we were able to produce intermittence of blood flow in the pulmonary vessels by the injection of small doses of epinephrine into the blood stream. These experiments are described later.

Richards and Schmidt have produced intermittence in the flow of blood in the glomerular circulation in the frog's kidney by gentle faradic stimulation of the sympathetic nerve fibers to that organ; also by small doses of epinephrine. It has not been possible to separate the sympathetic fibers to the cat's lung, but it is an accepted fact that such fibers occur in the lung of this animal. It is possible, therefore, that epinephrine may have acted upon these fibers to produce the intermittence of blood flow in our experiments.

Hooker (1911) and Richards (1914–15) have shown that the deprivation of oxygen will cause dilatation of arterioles and capillaries and Richards believes that oxygen lack is one factor in the mechanism of the intermittence in the glomerular circulation in the frog's kidney. It is difficult to believe that oxygen lack is a factor in the dilatation phase of the intermittent blood flow in the cat's lung, for the arterioles, with walls of capillary thinness, and the capillaries lie on the surfaces of the air sacs where oxygen is more accessible than at any other place in the body. Moreover, the production of asphyxia, as described later in this paper, did not cause arterioles or capillaries to open.

In several experiments we distended the lung so that its lower border moved 1 or 2 cm. downward, but slight or moderate changes of this degree did not interfere with the capillary or arteriolar flow. We feel confident that such changes in the insufflation pressure can be disregarded as playing any rôle in the intermittent changes in the capillaries noted

here. This observation is not in agreement with those of Hall who observed remarkable changes in the filling of minute vessels as a result of variable alveolar inflation. This might be explained by the difference in degree of inflation of the lungs. In our experiments, the lungs were inflated so slightly that the lower border moved no greater distance than it does in a full normal respiration. A closed chest enabled us to control this procedure accurately.

We were unable to determine the exact mechanism of the emptying of the capillaries, but certain observations which we have made on the pulmonary vessels agree closely with those of Krogh and Richards and Schmidt. In many experiments an arteriole was observed from which a number of capillaries on the surface of one air sac took their origin. Without obvious change in the flow in the parent arteriole, marked changes were observed in the blood flow in the capillaries. From such observations, both arterioles and capillaries appeared to empty themselves by contracting. Krogh and Richards and Schmidt, however, have shown that constriction of the entrance of the capillary may permit plasma only to enter, wash out the cells, and thus render the capillary invisible. Some of our observations lend support to this explanation. Repeatedly, we have noted the disappearance of a capillary except for occasional corpuscles which passed through it either rapidly or slowly in such a manner that they moved freely within the walls and showed no constriction in their shapes. At times one corpuscle would scarcely traverse the length of the capillary before another appeared. Between the corpuscles, however, the capillary was invisible.

In other experiments, some observations were recorded which at first glance suggested that pulmonary capillaries can contract with sufficient force to empty themselves. In experiment 9, while observing an air sac with three arterioles and eight capillaries, the rate of blood flow became much slower, then pulsatile in character, and finally the flow in seven of the capillaries stopped, although the blood remained in them. The eighth capillary and the arterioles continued to show pulsatile flow. The seven capillaries were gradually emptied without any pulsatile motion and disappeared. This observation might be interpreted as evidence that the capillaries emptied themselves by contracting, inasmuch as the pulsations were not transmitted to the blood column within them. On numerous occasions, however, we have observed pulsations in arterioles and a steady, constant flow in the capillaries arising from them. It is possible, therefore, that constriction of the entrances of the seven capillaries in the experiment just cited might have allowed the entrance of sufficient plasma to wash the cells out without showing pulsatile motion.

Still another observation is of great interest. In experiment 7, while watching the effect of an injection of pituitrin upon two arterioles and five

capillaries on an air sac, the circulation suddenly stopped in all the vessels. The blood remained stationary in both arterioles and in two of the capillaries. In the other three capillaries, the blood was gradually expelled peripherally. In this instance, the capillaries from the same parent arterioles behaved differently, but it is conceivable that the ostia of the first two capillaries were completely closed, while those of the three that were emptied were constricted sufficiently to permit the entrance of plasma only, which washed out the cells. It is also possible that these capillaries may have emptied themselves by contracting, but, inasmuch as we have not observed anything in the conduct of the capillaries which could be explained only by their ability to contract, our general conclusion drawn from these observations is that the behavior of the capillaries can be explained by the constriction at their ostia in such a manner as to permit "plasma skimming."

When capillaries branch or take their origin from other capillaries, the explanation of their actions becomes more difficult. We have observed alteration and intermittence of flow in such capillaries in many experiments. In these instances, it is possible that very slight changes of pressure in the parent arteriole might explain the intermittent flow. If "plasma skimming" is offered as the explanation of this behavior, it would imply contraction of the capillary at the point of branching from the other capillary.

The behavior in the pulmonary vessels thus far described has been observed in an unopened thorax. In a number of experiments, accidental destruction of the pleural windows afforded us opportunities to observe the vessels under the pressure relations brought about by the open window. In some such experiments we have observed intermittence of arteriolar and capillary blood flow. In several other instances, following the opening of the thorax the flow became steady and no intermittence was observed. The reason for the difference in reaction in some animals after opening the thorax is not clear. It is well known, however, that on opening the abdomen certain physiological reactions are changed.

The limitations of our method confined our observations to the lower edge of the lung. Hall, however, with a much greater surface of the lung exposed, observed various areas and found that the circulation in the vessels was essentially the same in all parts at any given time. MacGregor, on the other hand, observed no local changes in the smaller vessels in the presence of appreciable alteration in the total pulmonary blood inflow and outflow.

The method used in the above experiments also lends itself admirably to the microscopic observation of the action of drugs upon the smaller pulmonary blood vessels. When studying the action of drugs, the observer at the microscope was kept in ignorance as to the nature of the drug or substance to be injected or applied locally. Frequent injections and

applications of normal salt solution were carried out as control observations and no change was noted at any time as a result of the salt solution. All solutions for injection and local application were kept at body temperature.

Action of epinephrine.[7] Epinephrine was used in doses of various sizes and was injected intravenously or applied locally to the outer pleural window. The results were varied and inconstant, but certain effects were encountered with sufficient frequency to warrant their presentation.

The three effects most commonly observed were 1, increase in the velocity of blood flow; 2, intermittence of the capillary circulation, and 3, an increase in the number of active capillaries. Other less frequent effects were slowing or stopping of blood flow and reversal of the direction of the circulation, both of which were noted most commonly after large doses. All the effects noted, however, occurred after large and small doses. The range of dosage varied from 1.0 cc. of 1:10,000 solution to 0.2 cc. of 1:5,000,000 solution. (See figs. 9 and 10.)

In order to avoid the actions of epinephrine upon the heart and blood pressure and thereby eliminate the effect that these changes might have upon the pulmonary vessels, epinephrine was applied locally to the pleural window through which we were observing. It was felt that any changes in the vessels produced by this procedure would represent the direct action upon the vessels themselves. The following is an abstract from the protocol of experiment 10 (Feb. 7, 1927):

> At 4:22, while I was observing the vessels of an air sac, J. T. W. touched the external window with a piece of cotton which had been dipped in a solution. (This was a 1:100,000 solution of epinephrine.) The large arteriole emptied itself of blood and flow within it ceased almost instantaneously. This cessation of flow lasted 45 seconds and at the end of a minute flow was again resumed, at first in an intermittent fashion. In 1½ to 2 minutes the flow was once more constant in character. At 4:26 the velocity of the blood flow through the arteriole seemed greater than before the application. Needless to say, when the blood disappeared from the arteriole, the four capillaries arising from it also disappeared. (A. C. E.)

In this observation the large arteriole apparently constricted following the application of epinephrine. We do not feel sure of this, however, for there may have been a constriction at its ostium which permitted plasma only to pass through it. (The entrance of the arteriole was not visible in our field.) In either event, it would be rendered invisible. The systemic blood pressure remained unchanged throughout when the epinephrine was applied locally.

Repeatedly we have obtained evidence of constriction of vessels elsewhere in the circuit and not visible in our field of observation, as shown by

[7] We used adrenalin chloride tablets and the solution adrenalin chloride which contained chloretone and found no difference in the actions. Both products were put out by Parke, Davis & Co.

a cessation or marked slowing with pulsatile motion of the circulation. These results are similar to those of MacGregor. In only three experiments have we actually seen an arteriole contract and in each instance the vessel apparently closed. Even in these plasma may have been circulating. The fact that we were unable to see the vessel constrict, however, cannot be given too much weight, for the most commonly observed change after injections and local applications of epinephrine was an increase in the velocity of blood flow. After the injections this might be explained by the action of the heart, but following the local applications there was no change in heart rate or blood pressure and it is quite possible that slight constriction of the arterioles under observation escaped our notice. This is not at all unlikely when one recalls that the walls of the arterioles are invisible for the most part and that the calibre of the vessel is judged almost entirely by the width of the blood column. In two experiments, arterioles were probably constricted at their openings, for their walls remained visible and open and plasma was flowing through them as shown by the passage of an occasional red corpuscle. In one of these experiments changes followed an injection, and in the other, local applications of epinephrine.

Arterioles which had been closed and invisible opened with a normal flow after epinephrine, and both arterioles and capillaries have been seen to dilate following its use. (See fig. 10.) Whether the dilatation was due to an increase of pressure within the vessels or to action on them, we are not able to say.

The inconstancy of the results is striking as evidenced by the fact that following the use of epinephrine, in eighteen instances there was no detectable effect on the pulmonary vessels, and even the most common effects were not constant.

Action of pituitrin.[8] The action of pituitrin when injected or applied locally to the pleural window was much more constant than that of epinephrine. Constriction of the arterioles, ranging from a slight constriction after the injection of small doses to complete closure of the vessels after large doses, was the common effect observed. Partial or complete constriction of arterioles at their ostia was observed on several occasions and reversal of the direction of the circulation also occurred. The same results were noted when the drug was applied locally. Immediately after an injection or application, there was usually a transient increase in the velocity of flow, lasting a few seconds only, and followed immediately by a marked slowing or a complete cessation. Experiment 7, part 2 (see fig. 11) is a typical one.

As in the case of epinephrine, in a number of instances pituitrin failed

[8] "Infundin" of Burroughs and Wellcome was used in all experiments.

to produce any visible effect upon the vessels under observation. After the twenty-two injections of pituitrin, no effect on pulmonary vessels was noted in seven instances. Diminished velocity was noted after seven injections, flow was stopped completely four times, and capillaries only closed on four occasions. Reversed circulation was observed four times.

In experiment 7, part 2 (fig. 11) two injections of pituitrin were given in order to stop the circulation in the arterioles under observation. A large dose of epinephrine was then given for the purpose of raising the blood pressure in the pulmonary artery. Schafer and Lim (1919), Dixon and Hoyle (1929), and Berry and Daly (1931) have shown that a rise in the systemic or aortic blood pressure produced by epinephrine is accompanied by a rise in the pulmonary arterial pressure. The rise in aortic pressure in this experiment, therefore, was very probably accompanied by an appreciable rise in the pressure in the pulmonary artery. The actual rise of course is unknown, but the chart is presented to show that, if a rise in pulmonary pressure occurred after epinephrine, it was not sufficient to overcome the constriction produced by the pituitrin.

Nitrites. The nitrite group was administered intravenously as nitroglycerin or introduced into the ventilation system as amyl nitrite. Of thirty-nine administrations, no effect on the pulmonary vessels was observed after fifteen. In six instances, there was an increase in the number of capillaries. An increase in the velocity of blood flow in all vessels was noted in nine instances after the injection of a nitrite, while a definite slowing was observed on eight occasions. Flow in the visible vessels stopped entirely on two occasions and in one of these a reversal in the circulation occurred.

Other substances. Our eight observations upon the action of histamine on the pulmonary vessels are too few in number to justify any general conclusions. The most commonly noted action was a closure of arterioles and capillaries. In some instances evidence of constriction outside of our field of observation was obtained. This was shown by cessation of blood flow in the arterioles without obvious constriction. In one experiment, after a small dose of histamine, the velocity of flow increased, while in another with the same dose there was a decrease. At times no effect on pulmonary flow was noted.

Caffeine, digitalis, and atropine were given during the course of some of the experiments, but no noteworthy effects upon the pulmonary vessels were observed with the exception of an increase in the velocity of flow in the arterioles after atropine on two occasions.

Daly has recently summarized and discussed the lack of agreement in the reports of different workers concerning the action of epinephrine and other drugs on the pulmonary circulation. When we summarize our own results, we find them almost as variable as those in the literature. The

repetition of the same dose in the same animal may produce a different result.

One possible explanation of the variation in the action of the various drugs has been suggested by Daly, who advances the hypothesis that the difference in reaction is dependent upon the amount of the drug that reaches a given area in the lung. Our observations have shown that the number of arterioles and capillaries open in a given area of the lung at a given time may vary tremendously. Moreover, the blood flow within those vessels may be sluggish or rapid, and at times the blood column may even be at a complete standstill. This evidence would certainly indicate that a drug introduced into the blood stream would not reach every part of the lung in the same concentration and that some parts of the lung might escape its action entirely. It does not explain why the drugs do not act upon vessels showing active circulation.

Oxygen, carbon dioxide, nitrogen, etc. In the great majority of the experiments, air was used for the intratracheal inflation. Various mixtures of gas were tried in some of the experiments, however, in order to observe the effects, if any, upon the pulmonary vessels. Pure oxygen, nitrogen, carbon dioxide, and two mixtures of oxygen and carbon dioxide (O_2 90 per cent, CO_2 10 per cent and O_2 95 per cent, CO_2 5 per cent) were used. In general, we found that the blood pressure was maintained at a higher level and the animal's general condition remained satisfactory for a longer period of time when oxygen or the oxygen-carbon dioxide mixtures were employed instead of air. Sudden changes from air to any of the other gases, with the exception of nitrogen, produced no effect upon the rate of blood flow or the number of visible pulmonary arterioles and capillaries. We have six observations on the effect of substituting nitrogen for air in inflating the lungs. In each instance, the blood pressure soon began to fall and the circulation gradually ceased in all vessels, frequently showing pulsatile flow and then stopping altogether. All arterioles and capillaries were usually emptied. In one experiment, in which the cat was breathing when the nitrogen was started, the result was the same. In no instance, however, did we note any increase in the number of arterioles or capillaries showing circulation. Moreover, when the nitrogen was continued until the heart stopped, the vessels remained closed. We have noted similar behavior of the pulmonary vessels when death resulted from a failing circulation from any cause.

It seems worthy of remark that in only one of the experiments with nitrogen did we observe an asphyxial rise in the blood pressure, and even in that instance, it was so slight as to be scarcely noticeable. In all these experiments the condition of the animal before beginning the nitrogen was excellent.

In those experiments where asphyxia was the result of the inflation of the

lungs with carbon dioxide, there was no constant or striking change in the pulmonary circulation. As a rule, there was a gradual failure of the circulation similar to that observed following nitrogen inhalation.

Discussion. During the course of our work we have observed the opening and closing of arterioles and great variation of blood flow within them, as well as changes in calibre. Contractions of arterioles were infrequently seen but indirect evidence of contraction elsewhere in the pulmonary circuit was obtained repeatedly. In each instance the vessels were small, with walls approaching those of the capillaries in thinness, and they lay directly upon the surface of the air sacs. MacGregor's results were similar.

The wide variation of flow within the individual lung capillaries and the intermittence of flow in the arterioles and capillaries are more difficult to explain in the lung than in other structures. We made every effort to keep the lung as nearly as possible in its physiological state and we believe that our method with the thorax unopened does eliminate certain complicating factors which are introduced when the lungs are drawn out of the thoracic cavity or when they are perfused.

Our results are in agreement with several of the findings of Hall and MacGregor. On other points, however, we find our results at variance with theirs, and these have to do largely with the reactions and behavior of the blood vessels; Hall concluded that, provided the lung inflation and heart rate remain unaltered, the arterioles, capillaries, and venules do not show any changes in size and he obtained no evidence of disappearance and reappearance of active capillaries. It is our feeling that the difference in the two methods used will account for the different results obtained. Even with the lung in the chest and the pleura destroyed, the vessels failed to contract at times, so it may be that Hall's method of removing the lung from the chest, clamping it, and bringing it into contact with cotton and gauze will account for the failure of the vessels to react as they do within the closed thorax.

CONCLUSIONS

1. A method is described which makes possible direct microscopic observation of the smaller superficial pulmonary blood vessels and air sacs in the unopened chest of the cat.

2. The number of pulmonary arterioles through which the blood circulates at a given time is not constant. The rate and character of blood flow within them may change spontaneously or as the result of the injection of epinephrine and other substances.

3. Intermittence of blood flow has been observed in the arterioles.

4. The number of capillaries through which blood flows at a given time varies greatly and may represent a very small fraction of the total number of the capillaries in the lung.

5. The velocity of blood flow and the cell content of the blood may vary in capillaries arising from the same arteriole.

6. Intermittence of blood flow was commonly observed in the pulmonary capillaries, and we believe it to be the normal behavior of these vessels. It is also produced at times by epinephrine.

7. The changes in the capillary flow are probably governed by changes in the arterioles from which they arise and by slight changes in the pressure in the pulmonary circuit. No proof of contraction of the capillary walls was obtained.

8. The actions of epinephrine, pituitrin, nitrites, and histamine are described and the most commonly observed effects of these drugs are discussed.

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THE RÔLE OF THE INFERIOR MESENTERIC GANGLIA IN THE DIPHASIC RESPONSE OF THE COLON TO SYMPATHETIC STIMULI

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Thomas, in 1926, reported that stimulation of the mesenteric nerves in an excised nerve-muscle preparation from the small intestine produced a diphasic response, with inhibition as the primary phase. In 1931 Hendricks and Thienes confirmed Thomas' findings, and reported a similar response to adrenalin. In 1932 Templeton and Lawson observed strong longitudinal contractions in the large intestine of unanesthetized dogs, frequently associated with defecation, following intravenous injections of adrenalin. In the same year Barry described a strong motor phase following primary inhibition of the small intestine on stimulation of the major splanchnic in the dog.

These data stand in contrast with the well-known inhibitory effect of adrenalin upon isolated intestinal strips, and suggest that the motor phase is dependent upon mechanisms located outside the gut itself. Further, the data of Templeton and Lawson on the abolition of the adrenalin motor phase in the colon by atropinization or deep ether anesthesia suggest the rather obvious conclusion that the motor phase, whatever its causation, depends ultimately upon the same motor mechanisms in the gut as spontaneous motility, and may thus serve as an index to the state of such mechanisms.

The present investigation was undertaken in the hope that data on the causation of the second phase, of opposite sign to that of the primary phase, might throw light on the reversibility of effect of the gastro-intestinal nerves (McSwiney, 1931). If the response to these nerves is normally diphasic or polyphasic, a reversed response could be effected by relatively slight alteration in the phase relationships. This thesis is not established by the present data, but is strengthened by the finding that the duration, intensity, and distribution of the two major phases in the response are modifiable by procedures which may resemble in effect some of the better known reversing procedures.

METHODS. A detailed description of the special apparatus employed

has been given elsewhere (Lawson, 1934). Dogs fasted for 36 hours were lightly anesthetized with intravenous sodium barbital to the abolition of pseudo-affective responses, small supplementary doses being given during the experiment whenever necessary to keep the depth of anesthesia approximately constant. The colon was exposed through a midline incision, and stripped of its contents without irrigating. The belly walls were retracted to form a trough which was filled with 0.9 per cent salt solution kept at approximately body temperature. Nerves to be divided were placed at the beginning of the experiment in specially designed neurotomes with which they were cut without disturbing the viscera. Nerves to be stimulated were divided, and their peripheral stumps placed in modified fluid electrodes. Three enterographs of the lever type, modified for manometric recording, were attached longitudinally to the colon, each embracing a segment from 2 to 4 cm. long, in the following positions: no. 1, just distal to the root of the cecum; no. 2, midway between nos. 1 and 3, and above the level of the inferior mesenteric artery; no. 3, below the level of the inferior mesenteric artery. In the records presented, the serial records are numbered correspondingly. These segments are referred to in the text as proximal, middle, and distal segments, respectively. Tracing 4 in the records is a manometric recording of an air-inflated condom balloon, inserted through the anus and fixed at the level of the internal anal sphincter. The longitudinal enterographs were always attached with their fixed levers toward the anus, and were allowed to move freely with mass shifting of position of their segments so as to minimize interference in recording (Lawson, 1934).

Injections of adrenalin (Parke, Davis & Co.) were made into the exposed external jugular vein, the usual dose being 2 cc. of a 1:50,000 solution. A dose which was found during the control period to elicit readable responses was selected as a constant for the animal, which was repeatedly injected during subsequent procedures. Similarly, a constant faradic stimulus was selected for application to the nerves. This consisted of one minute of continuous tetanization with the secondary coil of the inductorium usually at 6 cm., the source of current being a battery of three dry cells with a high resistance in the primary circuit. The frequency and intensity remained constant within the limits of the apparatus throughout the experiment.

The constant stimulus, whether adrenalin or nerve faradization, was applied from three to eight times in each new condition of the animal, at intervals of 15 to 30 minutes. With the exception of the first thirty minutes to one hour of the tracing, and a few minutes following nerve sections, responses remained fairly constant so long as the experimental conditions were unchanged. There was, however, enough variation in many cases to make selection of a single representative response for each condition

difficult. Nor was it possible to express in simple numerical form the modifications observed. In many cases phase boundaries were not sharply defined, so that measurements of the tracings were inaccurate.

Average responses for each condition of the animal were constructed by transilluminating the record and tracing the separate responses on coördinate paper from a common time axis and a common base line. In this manner a superimposition of all the responses for that condition of the

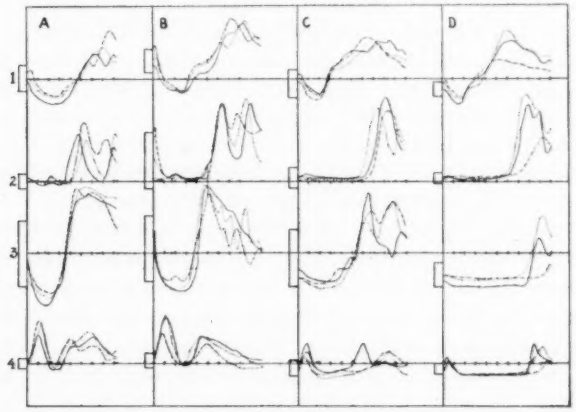

Fig. 1. Superimposed actual responses to adrenalin. Dog 19, weight 6.3 kgm. At the zero time axis in each section 2 cc. adrenalin 1:50,000 were injected intravenously. Three responses in each section are superimposed, the solid line being the first response, the fine broken line the second, and the coarse broken line the third. Average control motility limits are indicated by the rectangles erected on the zero time axis in each section (see text). The control motility levels of section A are projected as reference abscissae on which time is read in minutes from each injection. Time interval between adjacent injections about 25 minutes.

A. All nerves intact. B. After division of the rami of the inferior mesenteric ganglia. C. After division of both pelvic nerves. D. After division of both presacral nerves. The nerve sections are listed chronologically.

animal was obtained, as though the drum had been turned back each time to a common zero time axis. It was possible to construct, from the superimposed responses, curves which expressed the arithmetical average duration, intensity, and configuration of the various phases in each segment. The average responses for any two experimental conditions were then superimposed on coördinate paper for comparison. In somewhat conventionalized form, such individual average responses were used in the construction of simple arithmetical group averages, which are presented for the sake of brevity without an attempt at statistical correction. In

superimposing the individual responses a record was made not only of the immediate tone level of each segment at the time of the stimulus, but also of the average control motility, by marking on the zero time axis maximum and minimum levels reached by the tracing during the five minutes preceding each stimulus. Averages of these maxima and minima appear as limits of spontaneous motility during the control periods in each of the superimposed records presented. In forming the conventionalized group averages, a point midway between the average maximum and the average minimum was selected as the control motility level, and straight lines were drawn connecting this point, the point at which minimum tone was reached, the point at which tone began to rise, and the point at which the peak of the motor phase was reached. Beyond the peak of the motor phase, the smooth tone curve usually breaks into a series of contractions, the phases of which are so inconstant as to render the formation of a representative curve impossible. For this reason no data are presented on the duration or configuration of the terminal portion of the motor phase.

Observations. *Adrenalin.* The response to adrenalin has been described in detail for the distal colon (Templeton and Lawson, 1932). Serial longitudinal records of the entire colon show that the inhibitory phase is not of equal duration in all segments, and that the motor phase is not of equal amplitude or duration. Recovery from the inhibitory phase is usually seen first in the distal segment, somewhat later in the middle and proximal segments, with relationships between the two latter which are practically constant for the individual, but which vary from animal to animal (fig. 2A). The response of the sphincter appears as a primary motor phase with no readable latency at these drum speeds. It is almost co-extensive with the inhibitory phase in the distal segment. Frequently a second motor phase appears in the sphincter, at about the time of the distal motor phase. In intact animals the second motor phase usually follows the first without an interval (fig. 2A).

The response to adrenalin is practically constant, so long as experimental conditions are unchanged, in all segments. The greatest variability is usually observed in the response of the sphincter and of the distal segment (fig. 1).

After division of the rami of the inferior mesenteric ganglia, the duration of the inhibitory phase in the middle and the distal segment was almost invariably increased (figs. 1B, 2A, 3B, and table 1). This increase was usually most marked in the distal segment, so that recovery from inhibition now occurred at approximately the same time in all segments (fig. 2A), or in the order: proximal, middle, distal (fig. 3B). The height of the motor phase was usually increased in all segments. Group averages show a slight reduction in the height of the first motor phase in the sphincter, which was not observed in all animals. The second sphincter motor phase ap-

peared later than in intact animals, so that a definite interval occurred between the first and the second sphincter motor phase (fig. 2A).

When both presacral nerves (hypogastrics) were subsequently divided after the establishment of constant responses following ramisection, there was a further increase in the length of the inhibitory phase in the middle and distal segments, most marked in the distal segment, so that

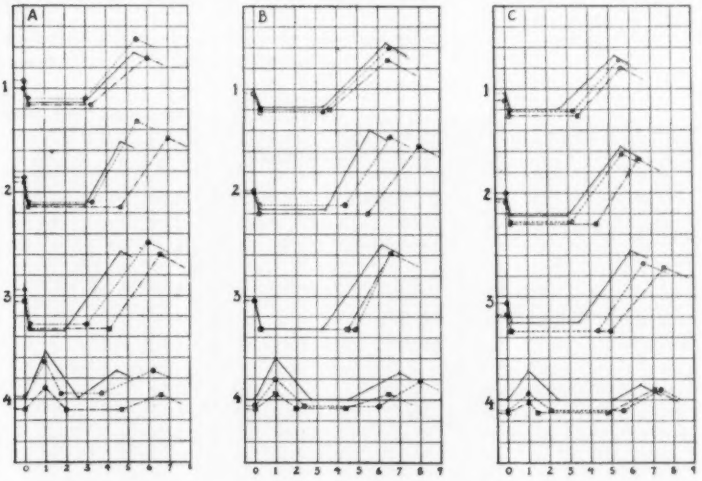

Fig. 2. Group average responses to adrenalin. The abscissae express time in minutes from the end of the injection, the ordinates centimeters of tracing height from base line.

A. Group 1 (11 animals). .———. All nerves intact (44 responses). ⊙-------⊙ After division of the rami of the ganglia (38 responses). ⊡— - —⊡ After division of the presacrals and the lumbar colonic nerve (36 responses).

B. Group 2 (9 animals). .———. After division of the rami of the ganglia (30 responses). ⊙-------⊙ After division of the presacral nerves (30 responses). ⊡— - —⊡ After division of the lumbar colonic (27 responses).

C. Group 3 (5 animals). .———. After division of the rami of the ganglia (14 responses). ⊙-------⊙ After division of the lumbar colonic (15 responses). ⊡— - —⊡ After division of the presacrals (15 responses). The nerve sections are listed chronologically. Cf. table 1.

the entire response resembled colonic peristalsis, a motor process apparently developing in the proximal segment, and progressing toward the anus into inhibited distal segments (figs. 2B, 3C). The height of the motor phase was reduced in all segments, as was the height of the first motor phase in the sphincter. The interval between the first and the second sphincter motor phase was increased (figs. 1D, 2B, and 3C). Di-

vision of the presacrals was effective in producing these modifications in animals with both pelvic nerves sectioned (fig. 1D).

In animals in which the picture of colonic peristalsis had been produced by section of the presacrals, subsequent section of the lumbar colonic nerve frequently destroyed the peristaltic sequence of the motor phase by

TABLE 1

The numbers in each column give number of animals whose average response shows the modification.

PREPARATION AND PROCEDURE	SEGMENT	INHIBITORY PHASE			MOTOR PHASE		
		Lengthened	Shortened	No change	Increased	Decreased	No change
I. Response to adrenalin							
a. Division of rami (12 animals). Intact controls	Proximal	3	3	6	8	3	1
	Middle	7	3	2	6	4	2
	Distal	11	0	1	6	4	2
b. Division of presacrals (10 animals). Ramisected controls	Proximal	3	3	4	2	6	2
	Middle	7	1	2	1	8	1
	Distal	7	1	2	2	8	0
c. Division of lumbar colonic (5 animals). Ramisected controls. Presacrals intact	Proximal	3	0	2	2	3	0
	Middle	1	0	4	2	3	0
	Distal	4	0	1	1	2	2
d. Division of both postganglionic trunks (18 animals). Ramisected controls	Proximal	11	1	6	2	9	7
	Middle	13	1	4	2	13	3
	Distal	14	1	3	3	11	4
II. Response to the lumbar colonic							
a. Division of rami (8 animals). Intact controls	Proximal	1	0	7	7	0	1
	Middle	3	1	4	4	3	1
	Distal	2	2	4	4	1	3
b. Division of presacrals (24 animals). Ramisected controls	Proximal	6	5	13	5	13	6
	Middle	10	6	8	5	14	5
	Distal	17	4	3	6	17	1

shortening the distal inhibitory phase and further lengthening the inhibitory phase in the middle segment (figs. 2B, 3D). In other cases there was no shortening of the distal inhibitory phase, but a similar disturbance in sequence by increasing the middle inhibitory phase beyond that of the distal. There was usually a further reduction in the height of the colonic motor phase, and in the height of both sphincter motor phases (fig. 2B).

Division of the lumbar colonic nerves in ramisected animals, with the presacrals intact, increased the duration of the inhibitory phase in the proximal and the distal segment, and reduced the height of the motor phase in all, including the sphincter (fig. 2C). There was usually little effect on the duration of the inhibitory phase in the middle segment. Subsequent presacral division in these animals now lengthened the inhibitory phase in the middle segment more than in any other. With both postganglionic trunks severed, the order of recovery from inhibition was usually proximal, distal, middle, regardless of the order in which the nerves were divided (fig. 2A and B).

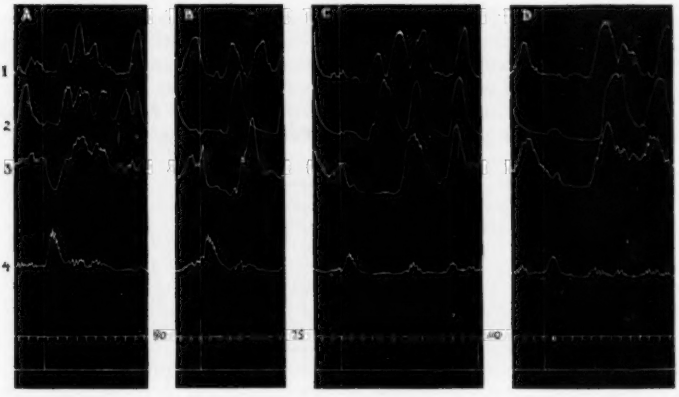

Fig. 3. The effect of division of the lumbar outflow upon the response to adrenalin. Dog 10, weight 7.2 kgm. At the ordinate erected in each section 2 cc. adrenalin 1:50,000 were injected intravenously.

A. All nerves intact. B. After division of the rami of the inferior mesenteric ganglia. C. After division of the presacral nerves. D. After division of the lumbar colonic nerve. Time in minutes.

The lumbar colonic nerve. The response to one minute of faradization of the lumbar colonic nerve resembles the response to adrenalin. The response is diphasic, the first phase consisting of a motor response in the sphincter, with depression of the entire colon. This is followed by a motor phase in the colon, frequently lasting as long as 20 minutes (fig. 4). Group averages showing distribution of phases are given in figure 5A.

Division of the rami of the inferior mesenteric ganglia usually increased slightly the height of the colonic motor phase, and reduced slightly the height of the first sphincter motor phase. There was no constant effect on the length of the inhibitory period in any segment (fig. 5A), although occasionally there was a slight and almost equal lengthening of the inhibitory phase in all segments (figs. 4B and 6B).

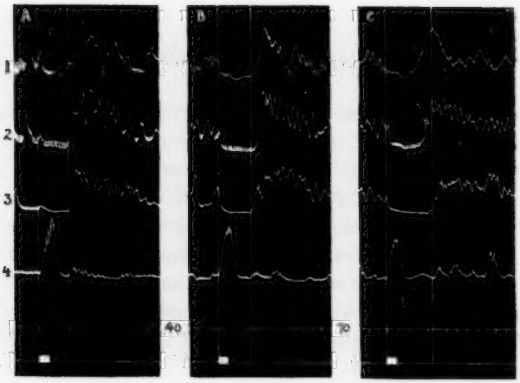

Fig. 4. The effect of division of the lumbar outflow upon the response to the lumbar colonic nerve. Dog 34. The signal in each section marks one minute of faradization of the peripheral stump of the divided nerve, with a constant frequency and intensity. Reference ordinates are erected at the beginning of each period of stimulation, and at the termination of the inhibitory phase in segment 3.

A. All other nerves intact. B. After division of the rami of the ganglia. C. After division of the presacral nerves. Time in minutes.

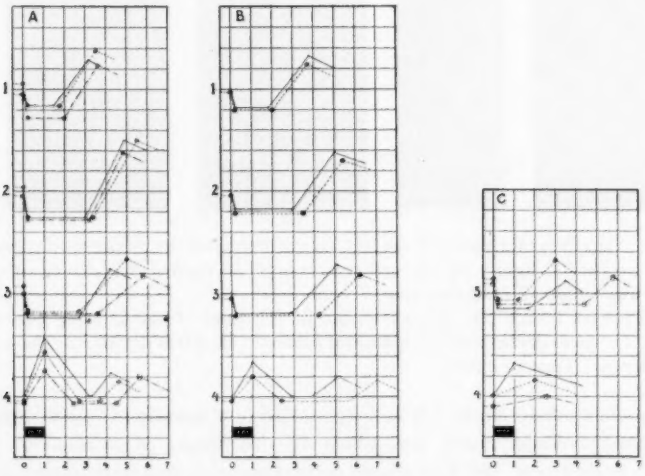

Fig. 5. Group average responses to faradization of the postganglionic trunks. Construction of figure as in figure 2.

A. Group 4 (7 animals). Faradization of the lumbar colonic. .———. All other nerves intact (24 responses). ⊙--------⊙ After division of the rami (22 responses). ⊡— - —⊡ After division of the presacrals (29 responses).

B. Group 5 (20 animals, including members of group 4). Faradization of the lumbar colonic nerve. .———. After division of the rami (57 responses). ⊙--------⊙ After division of the presacrals (64 responses).

C. Group 6 (4 animals). Faradization of the presacral nerves. Responses in segments 3 and 4 (see text). .———. All other nerves intact (14 responses). ⊙--------⊙After division of the rami (15 responses). ⊡— - —⊡ After division of the lumbar colonic (15 responses). The nerve sections are listed chronologically. Compare A and B with table 1.

Division of the presacral nerves, however, after ramisection, consistently reduced the height of the colonic motor phase, especially in the distal segment, and lengthened the distal inhibitory phase, so that the picture of colonic peristalsis was produced. The motor response of the sphincter was usually reduced (figs. 4C, 5A and B, and 6C).

Except for the response of the middle segment, the response to the lumbar colonic nerve was less constant for a constant condition of the animal than the response to adrenalin. The greatest variability was usually observed in the distal segment (fig. 6).

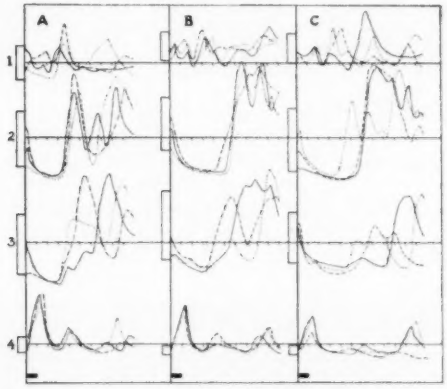

Fig. 6. Superimposed actual responses to faradization of the peripheral stump of the divided lumbar colonic nerve. Dog 24. At the zero time axis in each section a constant faradic stimulus lasting one minute (black signal) was applied. Construction of figure as in figure 1. Time interval between stimuli about 20 minutes. A. All other nerves intact. B. After division of the rami. C. After division of both presacrals. The nerve sections are listed in chronological order.

The presacral nerves. Faradization of the presacral nerves, combined in a single electrode, elicited variable responses except in the distal segment and sphincter. So little constancy, even in sign of phases, was observed in the response of the middle and proximal segments, that the construction of average responses was not possible except in the distal segment and sphincter (fig. 7). In the latter region the response resembled grossly the response to adrenalin and to the lumbar colonic nerve, except that the distal inhibitory phase was usually much shorter, and the motor response of the sphincter more prolonged (fig. 5C).

Following ramisection, the motor response of the sphincter was invariably reduced, and the motor phase in the distal segment augmented without significant change in the duration of the inhibitory phase. It was

usually not until after ramisection that any response was detected in the proximal and middle segments. After ramisection the spontaneous tone of these segments was usually markedly increased, and some response was nearly always elicited on stimulating the presacrals, but with no greater constancy than before (fig. 7B). Occasionally a delayed motor phase appeared in the middle segment, and still later in the proximal, as though propagated in an oral direction from the distal segment (fig. 8B).

Division of the lumbar colonic nerve in ramisected animals reduced the height of the sphincter response, increased the duration of the distal inhibitory phase, and reduced the height of the distal motor phase.

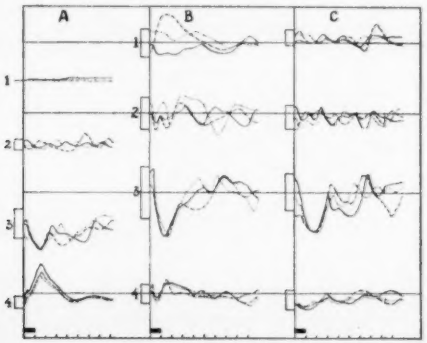

Fig. 7. Superimposed actual responses to faradization of both peripheral stumps of the divided presacral nerves. Dog 29. Construction of figure is the same as in figure 1 except that the control motility levels of section B are projected as reference abscissae.

A. All other nerves intact. B. After division of the rami. C. After division of the lumber colonic. The nerve sections are listed chronologically. Time interval between adjacent stimuli about 30 minutes.

Changes in the response of the upper segments were also noted, but their inconstancy does not permit a general statement (fig. 5C, 7C, and 8C). In one animal following lumbar colonic section the primary response in the distal segment was motor rather than inhibitory, with a delayed second motor phase following a depressed interval (fig. 8C).

The effect of stimulating the postganglionic trunks upon the response to adrenalin and the response to the lumbar colonic nerve. Preliminary attempts to demonstrate a modification of the response to adrenalin by preceding or simultaneous faradization of either nerve have not been completely successful. In a series of 4 animals, one was found in which adrenalin injected during the motor phase of the response to a preceding lumbar colonic stimulation produced higher motor responses in the colon

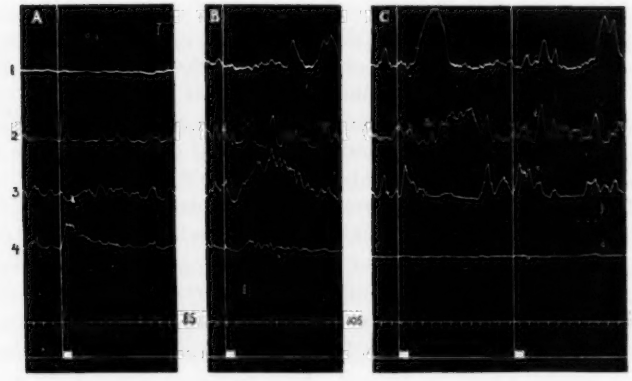

Fig. 8. The effect of division of the lumbar outflow upon the response to the presacral nerves. Dog 30. The signals mark application of the constant faradic stimulus to the peripheral stumps of both divided nerves. Reference ordinates are erected at the beginning of each stimulation.

A. All other nerves intact. B. After division of the rami. C. After division of the lumbar colonic (2 responses). Time in minutes.

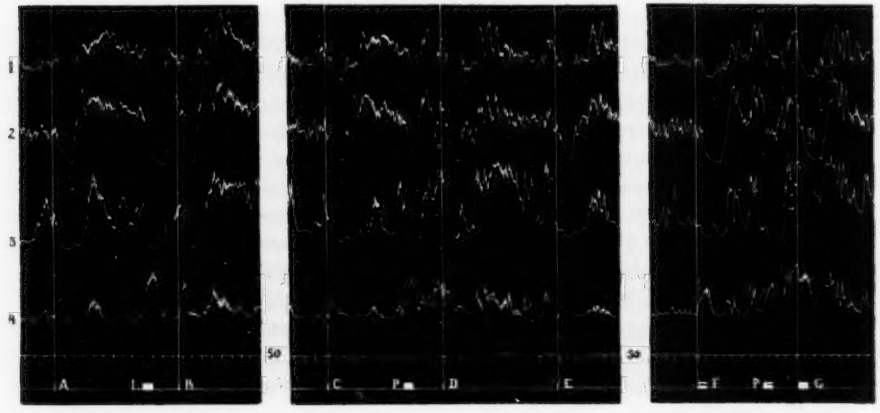

Fig. 9. The effect of postganglionic faradization upon the response to adrenalin, and upon the response to the lumbar colonic nerve. Dog 47, weight 8.3 kgm. *A*, *B*, *C*, *D*, and *E* are injections of 2 cc. adrenalin 1:50,000. *B* was given during the motor phase from a preceding faradization of the lumbar colonic, *L*, *D* during the motor phase from presacral stimulation, *P*. Both the lumbar colonic and the presacrals were divided. *F* and *G* are applications of the constant faradic stimulus to the lumbar colonic nerve, *G*, during the motor phase from a presacral stimulation, *P*. Time in minutes.

and the sphincter, with shorter inhibitory periods, than control injections (fig. 9). This modification was demonstrated without failure four times in this animal. In one animal of the series, adrenalin during a lumbar colonic motor phase produced much longer distal inhibition than control injections. In the other members of the group, there was no difference between the control responses and those elicited even during the height of the motor phase, or during the period of greatest depression.

Adrenalin injected during a presacral motor phase in three out of four animals consistently produced higher motor effects in the sphincter, and shorter distal inhibition than control injections, so that the sequence of recovery from inhibition was simultaneous or even antiperistaltic (fig. 9).

In five animals of a series of seven, the inhibitory phase in the distal and middle segments was reduced in duration, and the motor phase increased in height, when the lumbar colonic nerve was stimulated during a presacral motor phase. The height of the sphincter response was increased over that in control responses (fig. 9).

Discussion. The rôle of the preganglionic inflow to the inferior mesenteric ganglia (spinal rami) in all these responses is somewhat paradoxical. That the motor phase following any of these stimuli should be increased by section of the rami would be expected, since the tone and motility of the entire colon are increased (Garry, 1933). The paradox lies in the increase in the duration of the inhibitory phase. If the inhibitory phase is terminated by a true excitatory process, as the sudden recovery seems to indicate, any increase in the intensity of the excitatory process should shorten the inhibitory phase. That the excitatory process is intensified is shown by its increase in amplitude after ramisection. That its development is delayed must be due to an increase in the inhibitory process. Such an increase may be based on the unequal increase in the amplitude of the motor phase following ramisection. This phase, as well as spontaneous tone and motility, is most markedly increased in the proximal and middle segments. If intersegmental influences are active in distributing motor and inhibitory processes in the colon (Templeton and Lawson, 1931), it is conceivable that descending inhibitory impulses from the upper colon, with an intensity which is proportional to proximal motor processes, would be increased by ramisection, and would summate with the distal inhibitory phase.

Following the lead of Brown and Garry (1932), Carlson (1930), and others, the lengthened inhibitory phase following ramisection might be explained on the basis of the augmented tonus. However, the greatest increase in the inhibitory phase is observed in the distal segment, whose tonus is usually least augmented by the ramisection. Further, in a preliminary study, local excitation of a segment by pinching or direct faradization has usually decreased the local inhibitory phase, and augmented

the local motor phase. The use of tonus, per se, as an explanation of these modifications is not justified by the present data. Responses elicited in widely varying states of spontaneous tonus may agree in almost every detail.

Slight differences in the effect of ramisection on the response to adrenalin, presacral stimulation, and lumbar colonic stimulation are probably due to the fact that in the two latter types of stimulation preganglionic influences from large areas in the colon had already been removed by division of the postganglionic trunk for stimulation. This interpretation is justified by the fact that the remaining procedures produced almost identical modifications in the response to the three types of stimuli.

Garry (1933) reported an inhibitory influence of the hypogastric nerves on spontaneous motility after the spinal rami of the ganglia had been divided. The present experimental conditions are not sufficiently similar to his to warrant a comparison of the data. Frequently, however, for as long as 30 minutes following section of these nerves or the lumbar colonic in ramisected animals, tone and motility were augmented. Constant spontaneous motility was usually reached, however, at a slightly lower level than before. Even though the change in spontaneous tone level was in many cases insignificant or questionable, subsequent injections of adrenalin, or stimulation of the other postganglionic trunk, almost invariably gave responses differing markedly in intensity, duration, and distribution of phases, from the response of the ramisected control.

Both the presacrals and the lumbar colonic might be considered as supplying motor influences to the colon, differing in distribution, since the motor phase is decreased by section of either. The motor influence of the presacrals is felt most markedly in the distal segment and sphincter, while that of the lumbar colonic is exerted on both proximal and distal segments. A similar distribution of motor influences is seen on stimulating these nerves. The present data, in short, are interpretable on the assumption that the spontaneous activity of these nerves when removed from preganglionic influences is similar to the delayed effects of their artificial stimulation. That the peripheral effect of the spontaneous activity of either, furthermore, depends upon the integrity of the other, just as does the peripheral effect of either on artificial stimulation, is shown by such findings as that the effect of dividing the lumbar colonic depends upon whether or not the presacrals are intact.

It might be concluded that the rôle of the centrally isolated ganglion in the response to intravenous injection of adrenalin indicates an action of adrenalin upon the ganglion cells, a conclusion which is not incompatible with recent work (Dale, 1933). The almost identical rôle of the ganglion in the response to peripherally directed stimulation of a portion of the postganglionic outflow, however, argues against such a conclusion, since

there is no evidence that adrenalin-like substances liberated at the endings of these nerves enter the circulation in sufficient concentration to act upon unsensitized distant ganglion cells. Direct evidence against a humoral stimulation of the ganglion on peripherally directed stimulation of these nerves has been gathered in the present study, and will be presented in a later report.

An asphyxial or traumatic stimulation of the ganglion cells is possible under these experimental conditions, but would probably not remain constant for the long periods occupied by the experiment (6 to 10 hrs.). Stimulation of the ganglion cells by barbital is highly improbable, since additional doses of barbital given during the course of the experiment either produced no modification in subsequent responses, or (occasionally) a slight reduction in the motor phase and increase in the inhibitor phase, changes in the same direction as those produced by cutting the post-ganglionic fibres.

The possibility of recurrent centrifugal fibres in the postganglionic trunks, from other sources than the spinal rami of the ganglia, has been ruled out by demonstrating that cutting these nerves is still effective after both pelvic nerves have been cut, and the entire proximal colon denervated.

There remain the possibilities of ganglion cell automaticity, for which there is no evidence at hand, or a reflex activation of the ganglion cells. Tower (1933), in studying persistent action currents in the frog's mesenteric nerves following inflation of the gut, suggested the possibility of such reflexes through outlying ganglia. This possibility is strongly suggested by the present data, but is not in accord with current conceptions of the function of the sympathetic ganglia (Querido, 1924; Veach and Pereira, 1925; Bishop and Heinbecker, 1932; Orias, 1932; Knoefel and Davis, 1932; Rosenblueth and Rioch, 1933). That the suggested reflex activity of the ganglion cells is not an artefact such as that described by Langley and Anderson in explanation of the Sokownin reflex (1894) is shown by the fact that the rôle of the ganglia is almost identical in the response to adrenalin and in the response to artificial stimulation of the postganglionic trunks.

The demonstrated difference in the effect upon the response of cutting the preganglionic and the postganglionic portion of the outflow suggests such a difference in functional constituents as would be expected if such reflex arcs are present in the postganglionic portion. Differences in the response to artificial stimulation at the two levels have been recorded, and will be presented in a later paper.

The rôle played by the discharge from the centrally-isolated ganglia in these responses was first suggested by the observation that for a few minutes following interruption of a postganglionic trunk, responses were

similar to those during the control period. This would seem to eliminate the postganglionic trunks from a primary rôle in the distribution of the response, and to indicate a tonic effect upon peripheral structures. The data on combined stimuli lead to the same conclusion, and are comparable to those of McSwiney and Robson (1931) on the interaction of the vagus and the sympathetic on the stomach. Whether the rather large number of negatives in this series on combined stimuli means that chemical transmittors liberated in the periphery by the first stimulus (Rosenblueth, 1933) were almost exhausted at the time of the second stimulus, or whether it indicates that the greatest activity of the postulated reflex arcs occurs during the response, and hence cannot be replaced by a shortly preceding or simultaneous peripheral stimulus of short duration, is not known.

The distribution of constant and variable responses with the three stimuli suggests the distribution of primary and secondary effects. With adrenalin, responses are fairly constant throughout the colon, for a constant experimental condition. On stimulation of the lumbar colonic nerve, the most constant responses are obtained in the middle segment, while the response to the presacrals is inconstant except in the sphincter and the distal segment. Since this distribution agrees with the expected distribution of direct effects of each type of stimulation, it seems likely that the segments showing variable responses are, for the most part, outside the limits of distribution of the primary effects. Furthermore, since, according to this interpretation, even in the segments directly affected by a stimulus, disturbance of intersegmental relationships alters the response (e.g., modification of the response to adrenalin by interruption of the postganglionic trunks), it would have to be concluded that intersegmental relationships play a rôle even in segments directly affected by the stimulus.

Experimental demonstration of the part played by intersegmental relationships as suggested by these data will be offered in a later report.

The author wishes to express his appreciation to Dr. A. J. Carlson for criticising the manuscript.

SUMMARY

1. In barbitalized dogs small intravenous doses of adrenalin, and faradic stimulation of the peripheral stump of either the lumbar colonic nerve or the presacral (hypogastric) nerves produce primary inhibition of the colon, followed by a marked increase in tone and contractions. Both the motor and the inhibitory phase are seen in all segments following adrenalin, and following faradization of the lumbar colonic nerve. Following presacral stimulation, the two phases are obtained constantly only in the distal segments.

2. The height of the motor phase, and the duration of the inhibitory phase in all segments are altered by division of the spinal rami of the in-

ferior mesenteric ganglia, the motor phase being usually increased in height, and the inhibitory phase being prolonged in the lower segments.

3. After constant responses to any of the stimuli have been established following division of the rami, section of either the lumbar colonic or the presacral nerves produces further modification. This consists in a reduction in the height of the motor phase, and an increase in the duration of the inhibitory phase. Following section of the presacrals alone the increase in the inhibitory phase is most marked in the distal segment; following section of the lumbar colonics alone, in both the proximal and the distal segment. If the presacrals have been divided, subsequent section of the lumbar colonic may decrease the duration of the distal inhibitory phase.

4. The response of the internal sphincter of the anus to all these types of stimuli is usually triphasic: a primary motor phase coinciding with colonic inhibition, an interval, and a second motor phase at the time of the motor phase in the distal colon segment. Section of the rami or of either postganglionic trunk reduces the height of the first motor phase. The interval undergoes the same change as the distal inhibitory phase.

5. The height of the motor phase is increased in all segments, including the sphincter, if adrenalin is injected during the motor phase of a preceding postganglionic faradization, and the inhibitory phase is usually shortened. After a lumbar colonic stimulation the adrenalin inhibition is decreased in all segments; after a presacral stimulation the adrenalin inhibition is decreased in the distal segment.

6. The ganglion cells of the inferior mesenteric ganglia are shown by these data to be active independently of preganglionic influences, and to exert, through efferent fibres in the postganglionic stretch, influences upon which the peripheral effect of these stimuli depends. Since the demonstrated influence of the centrally-isolated postganglionic trunks consists in maintenance of time and intensity relationships between one segment of the colon and another during the response, it is tentatively concluded:

a, that intersegmental relationships play a part in the response of any segment to these stimuli, and *b*, that the value of the postulated intersegmental influences depends upon either an automatic or a reflex activity of the cells of the inferior mesenteric ganglia.

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THE FIBER CONSTITUTION OF THE DEPRESSOR NERVE OF THE RABBIT

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In the depressor branch of the vagus nerve of the rabbit there occurs a group of fibers with one prominent function, which traverses a pathway long enough to furnish a convenient nerve for physiological experimentation. That no other fibers than these should occur in this nerve would be so unique in the mammalian body, where even small nerve branches usually carry a variety of fibers to a given part, that we have felt a careful investigation to be in order before employing this nerve in certain functional studies for which it might appear to be peculiarly suitable. We have found that in general this nerve is neither simple, nor constant in its fiber content, and in the present paper we shall discuss the manner and the significance of its variability.

Animals were prepared under ether anesthesia for blood pressure recording from the right carotid, and for nerve stimulation and recording usually from the left depressor, sometimes from both. A nerve chamber was constructed (see fig. 1A) with 5 electrodes, two for stimulating, toward the head, one distal to this, grounded, and two more distal for leads to the oscillograph. Both vagi were cut, the sympathetic and depressor were separated from the vagus and usually mounted in the chamber without separation from each other, since stimulation of the peripheral end of the sympathetic has no appreciable effect on blood pressure, and the larger nerve mass dried less readily under experimental conditions. With the electrode chamber lying close to the body and covered with gauze, no other heat was found necessary. Condenser charges were employed as stimuli. Thresholds remained constant throughout a series of readings, and such a preparation could be stimulated for two hours without significant deterioration. Blood pressure responses however usually fell off after about an hour. Anesthesia by ether was kept as light as possible without movement. Many nerves were again recorded in a thermostat at 38°C. to check the data obtained

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on the nerves in the body. A mercury manometer was employed having one wide arm (fig. 1B) such that the change in level of the writing point read as 90 per cent of the change in pressure in millimeters of mercury.

The nerves were fixed in osmic acid, sectioned, and photographed at 1000 diameters. A chart was constructed consisting of circles of various sizes, and this was photographed on a process plate at such a reduction that the circles read in millimeters in the required sizes. Superposition of this "fibermeter" upon the nerve photograph allowed fibers to be read off directly in mu or fractions, each millimeter on the photographs and chart being equivalent to 1 mu. Areas were checked against the original section in the

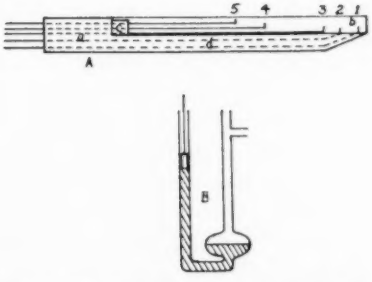
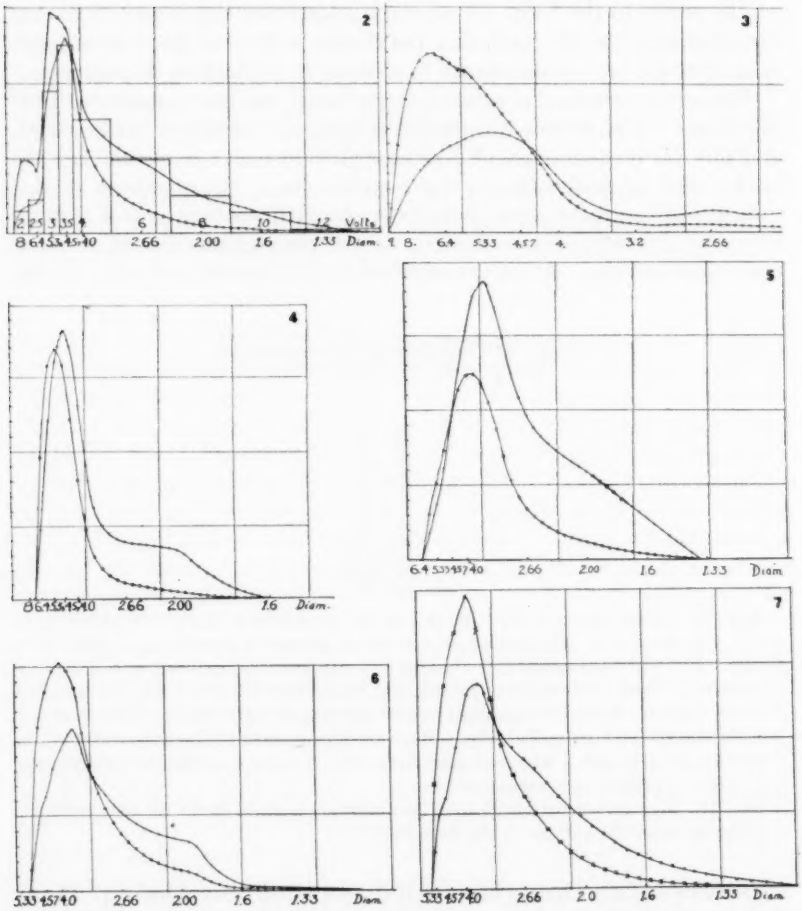


Fig. 1A. Nerve chamber for stimulating and recording of nerve still attached to body, side view, *1*, *2*, stimulating electrodes, *3*, grounded contact, *4*, *5*, lead electrodes. *3*, *4* and *5* are adjustable through holes in hard rubber base, *a*, clamped by set screws. Glass cover made of tubing split lengthwise fits over *c* and into grooves *d*, water sealed. End at *b* is plugged around nerve with wet cotton. Held in clamp at *a*, in contact with animal's body, and covered for warmth; ¾ inch diameter at *a*, 5 inches over all length. Electrodes are silver tips, *3*, *4* and *5*, soldered to stiff copper buss wire. Leads to apparatus shielded.

Fig. 1B. Manometer with bulb such that mercury surface under 100 mm. pressure is about 10 times the surface in the float limb.

microscope and any blurred fibers were drawn with a camera lucida. It was also found possible, and time saving, to project the slides in a dark hood onto a white paper, and measure the shadows directly at 1000 diameters with the "fibermeter" without photographing. A valuable feature of this technique is that each area may be brought into sharpest focus in spite of slight irregularities in the section. The fiber counts were plotted on a reciprocal scale, since they were to be compared with the corresponding action currents, and in the range of depressor fibers, threshold of stimulation is approximately the reciprocal of fiber diameter. An action current record was photographed for each blood pressure response, to step by step increases of stimulating voltage. These records were enlarged and their areas measured by a planimeter.



Figs. 2 to 7. Plots of number of myelinated fibers (dots) and cross sectional areas of fibers (crosses) against reciprocal of fiber diameter, the fibers being measured into groups by comparison with circles whose diameters are indicated below base line. Voltage of stimulation on figure 2 indicated above the base line, on the inference that voltage varies as the reciprocal of the diameter, the initial voltage being the threshold value for the nerve's potential. Method of plotting here indicated is explained in text.

If the fibers be arbitrarily divided into 3 groups, viz., above 5.3 mu, from 5.3 to 2.6 inclusive, and 2 mu, and below, the numbers of myelinated fibers run as follows: figure 2, 15, 243, 72, total 330; figure 3, 52, 150, 141, total 343; figure 4, 19, 295, 72, total 386; figure 5, 1, 240, 89, total 331; figure 6, 0, 216, 62, total 278; figure 7, 0, 124, 57, total 181.

Now if the voltage threshold for the first action current is divided by the reciprocal of the largest fiber diameter, a constant is obtained relating the two plots, of action current area against stimulus voltage, and of fiber number against size (figs. 2 and 8). If the fiber numbers are multiplied by the squares of the corresponding diameters, the resulting curve has a form similar to the curve of action current areas (for a somewhat different method of comparison, see Gasser and Erlanger, 1927).

Figures 2 to 7 present myelinated fiber counts of depressor nerves showing typical variations, the figures below the base line being the sizes of the circles with which the fibers were compared. These were so chosen that their reciprocals differed by a constant, except that with decreasing size, where the size intervals became too small to measure, simple multiples of the same constant were employed. For instance on the figures, the reciprocals of 10.66, 8, 6.4, and 5.33 differ by a constant value, those of 5.33 and 4 by twice this value, and of 4, 2.6, 2.0 and 1.6 by 4 times this value. Distances were laid off from center to center of the intervals resulting (illustrated on fig. 2), and the number of fibers whose sizes fell closest to a given measurement was divided by the corresponding distance to give the amplitude of a rectangle, whose area then measures the number of fibers involved. Through the resulting step curve a smoothed number curve was drawn such that the area between the sides of each rectangle, the curve and the X axis was the same as before, the total number of fibers being thus unaltered. Then for any convenient equal intervals on the base line, the number of fibers was multiplied by the square of the diameter of the middle fiber size in that interval, to give the fiber area curve. Allowing for small errors in smoothing the curve by this means, etc., the degree of fit between the form of this curve and of that of action potential area against voltage (figs. 8 and 9) is a test of the assumptions that area of potential varies as diameter squared, and threshold as the reciprocal of the diameter. The reason for choosing threshold instead of conduction rate as one parameter is that thresholds are more precisely measured, and the same applied to area of potential as compared to amplitude. There is also a possibility furthermore that if the fibers concerned have action currents which differ in duration (Blair and Erlanger, 1933), area may be a more significant index of fiber activity than amplitude.

The maxima of all area curves are naturally shifted toward the larger fiber end of the scale, as compared to the number curve, and the whole figure correspondingly skewed. For the same reason, any secondary elevation of the number curve will be relatively increased in the area curve if it falls ahead of the main peak, and decreased if it falls later. Several of these nerves show second elevations in numbers at about 2 or 2.6 mu, but no striking maxima in the area curves.

In figures 8 and 9 the plots of total action current area against voltage

(full line) may be compared with the plots of fiber area against the reciprocal of the diameter (dash lines) for the nerves of figures 2 and 3. The differential of the former curve, obtained by plotting the *increase* of area per unit increase of voltage, is also drawn (full line) together with the integral of the fiber area curve (dashes). These two nerves both contained fibers above 5.5 mu in such distribution as to give a specific distortion of the typical smooth distribution curve, and should thus be more critical for comparision of action current area with fiber area, since such irregularities give points of reference on the two curves. The nerve with the lesser first hump was recorded with too wide intervals of stimulus to show this, but

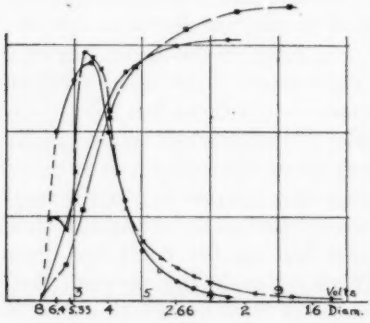


Fig. 8

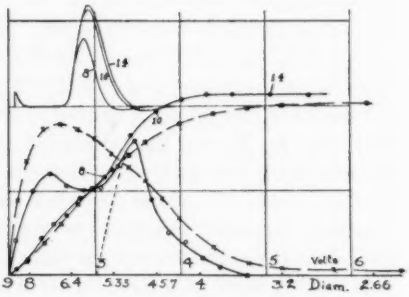


Fig. 9

Fig. 8. Plot of total action potential area against voltage (solid circles) and its differential, increase of area against increase of voltage (hollow circles); of fiber cross sectional area against reciprocal of fiber diameter (crosses) and its integral (squares) for the nerve of figure 2. The voltage steps were too large to detect the initial hump shown in the fiber counts.

Fig. 9. Same as figure 8 for the nerve of figure 3. Tracings of certain action potential records are placed above, enlarged to about the same dimensions, their numbers corresponding to the points numbered on the integral potential area curve.

the other showed a definitely double elevation. The latter nerve contained the largest fibers, and the greatest number of fibers larger than 5.5 mu of any nerve in the series. The dotted curve starting at 5.5 and passing to the second hump indicates the presumptive distribution of depressor fibers, the larger preceding group probably being a component usually passing through the vagus.

Allowing for the fact that when recorded in situ, the nerves were below normal temperature, and that by the time they were transferred to the thermostat for more precise recording after a blood-pressure experiment they had been dissected for some time, the agreements obtained in such plots are consistent with the assumptions that area of potential varies as area of fibers, within the narrow range of the main wave of the depressor;

a conclusion previously arrived at by Gasser and Erlanger (1927), for larger fibers, and more recently questioned by Blair and Erlanger (1933). The curves show, however, a consistent discrepancy in the small fiber end, in that the potential is lower than the area curve would predict. This might mean that these fibers produced less current, or that their thresholds were lower than assumed. We believe however that neither of these is true, but that the small fibers are for the most part motor autonomic fibers, with slower characteristics than the sensory fibers of similar histological aspect, and therefore are not activated in the same threshold range, and conduct more slowly to be recorded in a later wave.

The depressor nerve has two types of fibers histologically, myelinated and non-myelinated, the myelinated ranging in size from 10 to 1 mu, with a maximum of numbers between 4 and 2 mu. Physiologically it gives two, sometimes three action potential elevations. The first, and by far the largest, shows a conduction rate of 20 to 40 meters per second, the second (not always present as a discrete wave) about 5 meters per second, and a third, always very small, a rate of 1.5 meter or less per second, characteristic of non-myelinated fibers. The second is always well separated from the other two when present. Its conduction rate and refractory period correspond to the latter part of the "B" wave of the rabbit cervical sympathetic, which nerve generally contains no afferent fibers, and these depressor fibers are presumably motor. They have no discernible reflex effect on blood pressure or any other reflex effect. They are certainly among the smaller myelinated fibers of the nerve, are autonomic in type, and cannot be distinguished from the smaller sensory myelinated fibers histologically. They usually show as a characteristic elevation in the fiber size—number distribution, but only when numerous, as a second elevation in the fiber area curve. Non-myelinated fibers are always present, but in relatively small numbers, and a "C" wave cannot always be identified with certainty.

The rabbit cervical sympathetic contains typically only motor fibers, from 4 mu to 1 mu, its myelinated fiber action potential consisting of two parts of about equal size (Bishop and Heinbecker, 1932). The conduction rate of the second wave of the depressor when this is prominent enough to measure corresponds to the second part of the sympathetic potential. Its fibers should then correspond in size to the smaller group of the sympathetic; that is, some at least of the fibers around 2 mu in the depressor must correspond in properties to 2 mu symphathetic fibers. (Such potentials and fibers are also present in the vagus, but the situation there is confused by the presence of other than autonomic fibers.) The depressor 5 mu sensory fibers however have conduction rates about twice as high as the sympathetic 4 mu fibers, a ratio out of all proportion to the size-conduction rate relation in either sympathetic or depressor groups by themselves. The marked hiatus between the potential of the depressor associated with blood pressure

lowering and a considerably later separate wave that has no effect on blood pressure, further points to two functional groups of fibers, somatic afferent and autonomic efferent, overlapping in size range in this nerve, but with quite different physiological properties such as characterize these two types of fibers, somatic and autonomic, elsewhere in peripheral nerves (Bishop and Heinbecker, 1930).

Functionally, the fibers giving rise to the first potential (corresponding roughly to the sensory "B" wave of other nerves) always give upon stimulation, a fall of systemic blood pressure, with no immediate effect upon respiration or heart rate if the vagi are cut. (A delayed increase of respiration, with gradual onset, is presumably secondary to anemia of the respiratory center.) This depression, with maximal stimulation at a rate of 100 per second, varies in different animals between five and forty per cent. Often a rate of 50 per second will give the maximal effect. The number of myelinated fibers in the nerve varies between 150 and 600. Not all of the fibers corresponding to this first potential however depress the blood pressure. Many nerves contain also pressor fibers, and a few contain fibers which appear to be neither pressor nor depressor, but whose function has not been ascertained. When only depressor effects are obtained, the largest fibers in the nerve are usually about 5.5 mu with conduction rates of about 25 meters per second, and when larger fibers are present, depression of blood pressure does not occur at threshold as indicated by first appearance of the nerve's action current. Sometimes the first potential is distinctly double-humped, depression occurring coincidentally with the second hump of the potential. Comparison of such experimental data with fiber counts of the corresponding nerve indicate that all fibers above about 5.5 mu in size are other than depressor fibers, and below 5.5 mu pressor fibers also occur in some nerves. The maximum depression for any given rate of stimulation is attained at not more than four, usually within three times the threshold for depressor effects; though not necessarily at three times the threshold for first action current, when large fibers of low threshold give an action current without a corresponding depressor effect.

The fiber size of the typical depressor fibers of this nerve thus corresponds with that for pain and temperature sense in the saphenous (Heinbecker, Bishop and O'Leary, 1933), which latter nerve also contains within the same range fibers for vasometer reflexes. The fibers are slightly smaller than afferents from the lungs (Heinbecker and O'Leary, 1933) and smaller than the largest at least of the afferents that pass through the vagus from the heart. Both the vagus and the saphenous have depressor afferent fibers also in the "A" potential range, up to 12 to 15 mu in diameter.

A brief comparison with the vagus nerve, from which the depressor takes its origin, will furnish a striking contrast. The largest fibers in this nerve passing through the neck region are about 15 mu, and slow stimulation of

the central stump produces quite generally a fall of pressure, beginning at the threshold for the first action current. With stimulation somewhat above threshold, and of 50 to 100 per second, the depression due to slow stimulation is typically converted into a marked rise of pressure, a phenomenon previously reported by Gruber (1917). As the strength of stimulation is increased to reach fibers of the size range of the depressor nerve, the pressor effect increases. Thus the vagus has a predominance of large depressor fibers and small pressor fibers (characteristic also of the saphenous and other skin nerves) while the depressor has a predominance of small depressor fibers, and occasionally some large pressor fibers. From this point of view the nerves are complementary, although both may be complex. Disregarding the peripheral distribution for the moment, they show a complex but characteristic separation of a common supply of vasomotor afferents of various sizes.

This separation may be more complete or less so. It has been stated that the maximal depression obtainable from various animals varies from 5 to 40 per cent, while the fibers in various nerves vary in number from 150 to 600.

These values seem to be roughly related (fig. 11), in the sense that the larger the nerve, the greater the depression to be obtained from its stimulation. This to be sure seems reasonable; what is not so probable is that different rabbits would differ so widely in the efficacy of a characteristic vasomotor mechanism. We have usually studied the left depressor, but on numerous occasions when both were tested, opposite sides of the same rabbit have been nearly identical, thus ruling out a contralateral compensation for a weak effect. We therefore studied in a number of rabbits both vagus and depressor nerve vasomotor effects; it was found that when the depressor was effective (25 to 30 per cent depression) the vagus on strong stimulation gave a marked pressor effect, but when the depressor effect was weak, even fairly strong stimulation of the vagus gave marked depression (usually a deep fall followed by a rise). Frequently it has been found that vagus stimulation at 100 per second resulted in a greater fall of blood pressure than did stimulation of the depressor itself, although this was followed by a rise during stronger stimulation, and in one case where depressor stimulation was almost ineffective, the *depressor* effect of vagus stimulation overpowered the vagus pressor effect, so that only after cessation of stimulation did the pressure rise above the normal level.

From these results we conclude that while the depressor branch of the vagus nerve is typically the preferred pathway for depressor fibers from the aorta, the vagus itself is an optional pathway, and that other than depressor fibers (presumably also from the heart region) can pass over the depressor, although typically traversing the vagus. Karasek (1933) reports responses of pressor fibers in the depressor, and both he and Braueucker (1923) hold that vagus (and sympathetic) fibers may be present. The number of such

fibers, both myelinated and non-myelinated, varies from rabbit to rabbit, as can be observed both from the characteristic action currents, and from the appearance of osmicated cross-sections. In other mixed nerves, the myelinated autonomic and the non-myelinated fibers are grouped together in islands, the interstices of the myelinated axons showing as a grey or brownish background in osmic acid, which the pyridine silver stain shows to contain non-myelinated fibers; while in areas containing only somatic fibers, the interstices are clear. The depressor may or may not have such areas clearly defined. In some nerves a crescent at one side may contain them, in others the whole nerve. Some of the largest depressors of which we have sections show almost none, and there seems to be no correlation with nerve size. Several nerves sectioned high in the neck, and showing either two branches (one from the vagus, and one from the laryngeal), or two distinct areas representing these two branches joined to the common trunk, have autonomic areas in one component, but less in the other. Not all nerves have this double source however, and we have not traced the pathway of such fibers. The point here is that other than depressor fibers vary in numbers in the depressor nerve. In view of the alternate pathway for such fibers via the vagus itself, we infer that the variable number of fibers, either motor or sensory, does not connote a variable efficacy of the mechanism involving them for the animals as a whole, but merely a variable distribution in vagus and depressor, of a possibly constant total number of fibers.

In two sympathetic nerves (the sympathetic and depressor were usually prepared together), sections show fibers larger in diameter than the typical 4 mu or smaller autonomic myelinated fibers which this nerve always contains. These fibers were distributed chiefly in one crescent along the side of the nerve, the rest of this fascicle and other fascicles, of which several are usually present, having the typical sympathetic aspect. In these preparations a separate depressor was also found. Presumably these nerves approach the arrangement typical of the cat, where only occasionally is the depressor separate, while the sympathetic carries afferent fibers in variable numbers.

Out of 40 rabbits on which blood pressure was recorded, sections of at least one depressor nerve were made from 32, and all the fibers were counted in each of 15, by size groups, as indicated in figure 10. In osmic acid preparations, the areas of each nerve cross section containing myelinated fibers, excluding prominent connective tissue septa and vacant spaces due to stretching of the paraffin section, were measured with a planimeter after drawing in a camera lucida. In figure 10 the sum of all the areas of nerve fibers in each of the 15 nerves counted (x axis) is plotted against the planimeter value of that nerve's cross section, and the straight line through the origin is the nearest fit to these values (solid circles). For those nerves not

counted, their planimeter measurements were laid off on the y axis, and horizontal lines drawn to interesect the graph. These points give the approximate fiber count areas for the nerves not counted. The values of fiber area of this figure are plotted against the observed per cent depression of blood pressure on maximal stimulation of one depressor nerve at 100 per second (fig. 11, dots). It will be observed that the blood pressure values scatter considerably, but show some tendency to vary with the size of the nerve. On the same graph (crosses) are plotted corresponding points when the areas of fibers of over 5.5 mu in diameter are subtracted from the totals of such nerves as have the larger fibers. The nerves containing numerous large fibers prove to be the larger nerves with disporportionately low depressor effects, and this procedure reduces the scattering. There is no

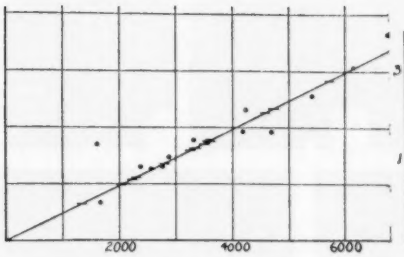


Fig. 10

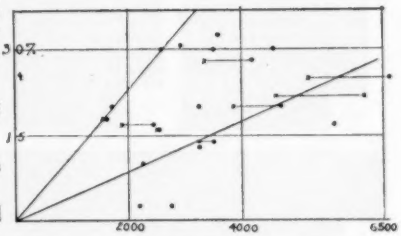


Fig. 11

Fig. 10. Area of nerve cross section (ordinate) against the sum of the areas of myelinated nerve fibers. The 15 nerves whose fibers were counted and measured are represented as dots, the straight line through the origin being the nearest fit. Other nerves measured but not counted located by intersection of horizontal line through nerve area value on y axis with the diagonal determined by counted nerves.

Fig. 11. Dots, per cent depression of blood pressure on maximal stimulation of one depressor nerve at 100/sec., against total area of myelinated fibers from graph of figure 10. Crosses, area of certain nerves containing fibers larger than 5.5 mu corrected by subtracting from the total area the area of those larger fibers.

way of correcting for fibers of smaller diameter that are not depressor fibers, which these and other nerves probably contain, and these may account for some of the scattering; pressor fibers in the same threshold range as depressor fibers would counteract the effects of the latter when both were electrically stimulated.

Pressor effects are recognized by an initial rise on weak stimulation (fig. 12) with a short latent period, followed by depression at anything above threshold; or by a fall followed by a partial return during stimulation. In the first case we infer that fibers as large as, or larger, than any depressor fibers are present, and in several cases, as evidence of this, the threshold for pressor effects has been above the threshold for first action current. In

the second case pressor fibers presumably occur in the same size range as depressor, and cannot therefore be detected anatomically. It is significant that when initial pressor effects occur near threshold, stonger or higher frequency stimulation may abolish them. This occurs in spite of the fact that the depression even with such stimulation still shows a latent period long enough to allow pressure effects to have taken place. This could be explained if the pressor center reacted, (as it is supposed to do, Bayliss, 1923) to both pressor and depressor fiber stimuli, but antagonistically; and if the inhibition as well as the stimulation of the pressor center were more prompt than the stimulation of the depressor center. If both are sup-

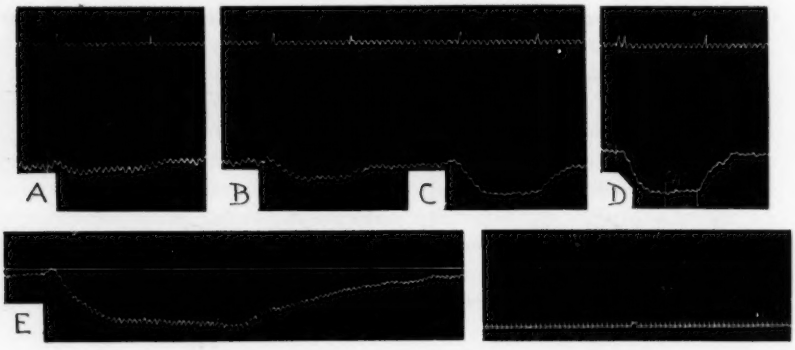


Fig. 12. Pressor effects from stimulating the depressor nerve, A, 6 volts, condenser charges, 24/sec. B, 10.5 volts, 24/sec. C, 10.5 volts, 48/sec. D, 22 volts (max.) 48/sec. Note that with increasing strength and frequency, the initial pressor effect is abolished, without, however, altering the latent period of depression. E, another preparation, maximal stimulation at 64/sec. has not abolished the initial rise. Other nerves give a partial rise toward the initial level after fall, without this initial rise, presumably due to smaller pressor fibers stimulated only at strength that activates many depressor fibers. Time in seconds. Respiration and time of stimulus above.

posed to act mainly via the same sympathetic pathways, the difference in latent periods must be central rather than peripheral, but we feel that these points cannot be settled on the basis of present evidence.

SUMMARY

A combined histological and physiological study of the depressor branch of the vagus nerve of the rabbit indicates that this nerve is not purely sensory, but contains vagus efferent fibers, both myelinated and non-myelinated, and that of the afferent fibers, not all have a depressor effect.

The size range of depressor fibers lies between 5.5 and about 2 mu. Fibers larger than this, up to 9 or 10 mu, occasionally present, are sometimes

afferent pressor fibers, sometimes of other undetermined function. Autonomic myelinated fibers and the smaller afferent depressor fibers are indistinguishable anatomically by present methods, but differ in their physiological characteristics. Pressor and presumably other afferent fibers may be present in the size range of typically depressor fibers, as well as in the larger size group.

The maximum depression obtainable from stimulation of one depressor nerve varies widely from rabbit to rabbit; the number of myelinated fibers in the nerve varies from 150 to 600; there is some correspondence between the size of the nerve and the maximum depression obtainable.

The vagus has many depressor fibers larger than those in its depressor branch while the majority of its pressor fibers lie in the size range of depressor fibers in the depressor nerve. Evidence is presented that depressor fibers (presumably from the heart region) are variably distributed between vagus and depressor nerves, accounting in part at least for the variability in numbers of fibers in the depressor.

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REDUCTION OF OXYGEN CONSUMPTION DURING CARDIAC INHIBITION[1]

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A reduction in the oxygen consumption by the beating heart under the inhibitory influence of the vagus nerves has been well established for mammals by Barcroft and Dixon (1) and by Wolfsohn and Ketron (2), and for the frog by Bohnenkamp and Friedman (3). This is to be expected when one considers the effect of the vagi on work done and tension developed by the cardiac muscle, since Clark and White (4) have shown a reduction in oxygen consumption by procedures which reduce the strength of contractions. There still remains the problem of determining whether vagal inhibition can reduce the oxygen consumption below the resting level, for arrest is not inhibition since a heart at rest can still contract when stimulated, and such physiological responses are diminished or extinguished by vagus inhibition. The electrical positive variation demonstrable in the resting heart upon vagal stimulation (5, 6, 7, 8) points to a definite physicochemical change underlying the active inhibitory response. That it may well be associated with changes in oxygen utilization by the heart was suggested by the fact that inhibition of the ganglion cells of the Limulus heart is accompanied by a reduction of CO_2 production (9) and oxygen consumption (10) and a preliminary study with the turtle's auricle (11) indicated that a similar situation is to be met with in cardiac inhibition.

METHODS. In order to test this possibility the hearts of turtles were removed from the body along with the entire cervical length (10 cm.) of one or both vagus nerves, including only a minimal amount of circumcardiac tissue. The ventricle was excised leaving a vagus-auricle preparation which could be inhibited. The heart was washed free of blood with Ringer's solution followed by pericardiac fluid, then after drying the outer surface of this preparation a strong solution of acetylcholine of methyl acetylcholine was carefully applied to the sinus venosus alone by means of a tiny pointed applicator; meticulous care was taken to avoid touching any of the solution to auricular tissue. This application was continued and extended until microscopical examination gave assurance that no vestige of beat of the

[1] This research was financed by a fluid research fund granted by the Rockefeller Foundation.

pace maker was possible. Such preparations remained quiet for several hours although the arrested auricles would respond to mechanical and electrical stimulation and by stimulation of the vagi could be inhibited to complete loss of response. The oxygen consumption and carbon dioxide production could be determined in the Thunberg-Winterstien or Parker apparatus, but for the vagus effect we adopted a modified Barcroft-Warburg micro-respiration chamber of 17 cc. capacity and calculated the volume changes from manometric readings of the contained Brodie solution by the method of Warburg (12) which gives the volume at standard conditions. The maximum surface of the auricles was exposed on a paraffined metal shelf and either one or both vagi were threaded through a length (4 cm.) of a small tube, the upper end of which projected above the surface of a constant temperature water bath. The tube contained a seal-mixture of kaolin and vaseline and the end of the tube was capped with a thin rubber membrane containing a pinhole through which the nerves could be drawn and laid on the stimulating electrodes. Potassium hydroxide in the chamber absorbed all carbon dioxide formed.

Before immersion in the water bath the chamber of the apparatus was filled with oxygen in order to secure adequate diffusion pressure into the thin auricular tissue. This is a desirable procedure although it was found that the oxygen consumption from air was almost as active as from oxygen. This fact assured us that the tissue was thin enough to acquire an adequate oxygen supply, which is in keeping with the experience of Clark and White (4).

Temperature regulation of the water bath was easily secured as all experiments on the effects of the vagi were conducted at room temperature. Since some of the experiments were run during the early summer months the temperatures recorded are relatively high. Temperature equilibrium between the bath and the interior of the apparatus was quickly acquired, fifteen minutes sufficing. Thereafter manometric readings were made every five minutes and a check blank apparatus was likewise read to assure an adequate control from which corrections of readings could be calculated. The nerves were stimulated with the faradic stimuli from an induction coil or by rhythmic discharges of a condenser.

Experiments. The oxygen absorption by the resting preparation usually continued at a constant rate for two hours or more, in some instances for more than four hours, so that a plat of the readings taken every five minutes formed a straight line, the later readings showed a gradual progressive decrease in oxygen consumption. Most of our experiments were concluded within the first hour. The oxygen consumption is computed in cubic centimeters per gram per hour, the weight used being the moist weight of the auricular tissue, after subtracting the oxygen used by the extraneous tissue unavoidably included in a functioning preparation.

This latter amounted quite uniformly to plus or minus 5.4 per cent of the total oxygen metabolism and was, of course, unaffected by the action of the vagus nerves.

The results of a series of experiments are presented in table 1 in which each figure for resting oxygen consumption is calculated from observations over a twenty minute period and in table 2 in which five minute periods form the basis for the calculation, also the effects of stimulating a

TABLE 1

Reduction of oxygen consumption when inhibiting turtles' auricles by stimulating one vagus nerve

The figures represent cubic centimeters per gram per hour (moist weight). Calculations based on twenty minute periods.

TEMPERATURE	RESTING AURICLES	DURING INHIBITION	PERCENTAGE DECREASE	POST VAGAL
°C.	cc.	cc.	per cent	cc.
28	0.330	0.300	9	0.314
31	0.203	0.127	37.5	0.184
32.5	0.282	0.099	65	0.325
30	0.280	0.234	16.5	0.325
28	0.345	0.230	33	0.375
26.5	0.237	0.217	8.5	0.249
31	0.150	0.081	46	0.162
31	0.160	0.122	24	0.229
28.5	0.433	0.289	33	0.380
31.5	0.370	0.270	27	0.346
28.5	0.250	0.152	39.5	0.215
29	0.216	0.137	36.5	0.250
21	0.207	0.146	29.5	0.207
22	0.148	0.110	25.4	0.160
25	0.220	0.194	12	0.211
25	0.200	0.150	25	0.193
24	0.133	0.083	37.5	0.158
24.5	0.226	0.158	30	0.248
22	0.214	0.107	50	0.320
22.5	0.160	0.064	60	0.200
Average.....	0.238	0.1635	31.3	0.252

single vagus for the corresponding period and a post vagal run as a check to determine whether the rate of oxygen returned to a resting level after nerve stimulation. It is a general experience that similar tissues from different individual cold blooded animals of a given species show different levels of oxygen consumption per gram of tissue weight, and our experiments run true to form in this regard—a minimum of 0.133 cc. per gram-hour (24°) and a maximum of 0.433 per gram-hour (28.5°). At lower temperatures the

rate of oxygen utilization by different hearts is different but of the same order of magnitude, and in general lies around 0.2 to 0.25 cc. per gram-hour. At higher temperatures the tissues of different animals show more variation, although in general the consumption of oxygen is greater than at lower

TABLE 2

Reduction in oxygen consumption by resting turtles' auricles when inhibited by stimulating one vagus nerve. Cubic centimeters per gram per hour (moist weight)

Calculations based on five minute periods

TEMPERATURE	RESTING AURICLES	VAGUS STIMULATION	PERCENTAGE DECREASE	POST-VAGAL REST
°C.	cc.	cc.	per cent	cc.
24.5	0.126	0.101	19.84	0.138
24.5	0.144	0.07	51.38	0.169
24.5	0.125	0.07	44.0	0.151
24.9	0.201	0.082	59.2	0.176
24.9	0.164	0.119	27.43	0.188
24.3	0.140	0.104	25.71	0.157
23.1	0.201	0.121	39.8	0.173
24.7	0.26	0.155	40.1	0.275
24.7	0.225	0.155	31.1	0.268
23.0	0.283	0.121	57.24	0.294
23.0	0.285	0.199	30.17	0.328
23.0	0.324	0.208	35.8	0.276
23.0	0.273	0.225	17.6	0.250
23.0	0.253	0.173	31.62	0.285
23.0	0.284	0.200	29.57	0.276
24.0	0.278	0.194	30.21	0.274
24.0	0.284	0.206	27.46	0.278
24.0	0.278	0.194	30.21	0.278
27.0	0.275	0.212	23.0	0.30
27.0	0.262	0.225	14.12	0.27
27.0	0.256	0.228	10.94	0.259
24.0	0.218	0.1935	11.24	0.226
24.0	0.210	0.1695	19.28	0.218
24.0	0.218	0.1775	18.57	0.234
22.0	0.1324	0.1135	14.27	0.136
22.0	0.155	0.1135	26.77	0.1627
22.0	0.1287	0.095	26.34	0.1325
22.0	0.125	0.099	20.8	0.1325
Average.....	0.2181	0.1544	29.2	0.2251

temperatures. Unpublished data show that any individual heart follows the usual temperature coefficient for changes in oxygen consumption.

Each experiment in tables 1 and 2 demonstrates that there is a definite decrease in the rate of oxygen consumption during the period of vagus inhi-

bition, the decrease varying from 8.5 per cent to 65 per cent or even as much as 84 per cent over a period of stimulation. These differences presumably are to be accounted for by the differences in the functional integrity of the preparation which varies with the trauma due to their removal from the animal, furthermore due allowance must be made for differences in the strength of the stimuli used—a matter of much significance which was not controlled in our earlier experiments but which is considered later in this communication.

Control series. In evaluating the results of the above experiments it is essential to be assured that stimuli applied to the vagus nerve do not develop heat, which by conduction into the apparatus along the nerve, would cause sufficient expansion to check the manometric change due to oxygen consumption and thus give a false value to the readings which might be interpreted as a reduction in oxygen consumption. A number of the heart vagus preparations were so badly injured in their removal that vagus stimulation made no alteration in the course of the curve of oxygen consumption; such "failures" however served as control experiments showing that there was no physical conduction of heat along the nerve which had any perceptible effect on the manometer. In a second group after demonstrating the physiological effects of inhibition the vagi were crushed with a complete abolition of all effects of stimulation. Finally in coöperation with Dr. H. S. Wells, the preparation was placed upon a series of thermopile junctions mounted on the shelf of the oxygen chamber and connected with a sensitive galvanometer capable of detecting a change of 1.2 microcalorie per second for the amount of cardiac tissue involved. There was no perceptible heating effect on stimulating the vagi which had been crushed close to the heart, even when stimulated for five minutes by 18 discharges per second of a 0.1 microfarad condenser charged at forty-five volts. Since the vagal effects on oxygen consumption recorded in the preceding section involve calculated heat changes (assuming they are accompanied by heat changes) which might be fifty or one hundred times the detectable change, there can be no question that all changes recorded for vagus stimulation were due to decrease in oxygen consumption and not to any heating effects from extraneous sources.

Degree of inhibition. There is a direct correspondence between the strength of vagal stimulation and the depression of oxygen consumption as is brought out in the experiments summarized in table 3 in which for the strengths of stimuli chosen the stronger produces on the average a 50 per cent greater decrease than the weaker. Two specific effects are probably involved in the results under consideration, the first of these is the degree of inhibition induced in the tissue innervated, for the results in table 3 were obtained by the stimulation of a single vagus nerve—the right. The other concerns the amount of tissue subjected to inhibition; this is shown by the

fact that if the reduction of oxygen used is demonstrated by moderate stimulation of one vagus there is further reduction in the rate of O_2 consumption by laying the second vagus across the electrodes, although the further reduction rarely equals that due to the action of one nerve alone. Owing to the preponderant ipsolateral distribution of the vagi to the turtle's auricles (13) stimulation of the second vagus adds to the muscular tissue subjected to inhibition. The results obtained by stimulating one vagus with a series of mild faradic shocks and then laying the second vagus across the electrodes are presented in table 3 and need no comment other than to direct attention to the fact that weak stimuli were used to produce only

TABLE 3

Stronger cardiac inhibition causes greater decrease in oxygen consumption. Cubic centimeters per gram per hour (moist weight)

Calculations based on five minute periods

TEMPERATURE	RESTING AURICLES BEFORE INHIBITION	WEAK VAGUS STIMULATION	PERCENTAGE DECREASE	STRONGER VAGUS STIMULATION	PERCENTAGE DECREASE	POST INHIBITORY PERIOD
°C.	*cc.*	*cc.*	*per cent*	*cc.*	*per cent*	*cc.*
24.5	0.180	0.135	25.0	0.045	75.0	0.220
22.0	0.210	0.103	51.0	0.034	84.0	
22.0	0.320	0.235	26.5	0.160	50.0	0.268
21.0	0.253	0.158	37.4	0.1265	50.0	0.220
21.0	0.202	0.155	23.2	0.1265	37.0	0.221
21.0	0.190	0.127	33.0	0.064	66.3	0.210
24.5	0.126	0.101	19.84	0.07	44.44	0.144
24.5	0.1505	0.112	25.58	0.103	31.56	0.164
23.0	0.273	0.225	17.6	0.173	36.63	0.285
24.0	0.218	0.1935	11.23	0.1695	22.24	0.234
22.0	0.133	0.113	15.03	0.095	28.57	0.155
22.0	0.208	0.1745	16.1	0.1575	24.27	0.20
Average ..	0.2053	0.1526	25.66	0.1103	46.27	0.211

mild degrees of inhibition and reduction in the oxygen consumption, thus making possible the increment of effect when the second vagus was stimulated.

Repeated experiments demonstrated the fact that the greatest inhibitory effects upon the rate of oxygen consumption might be expected in the first five minutes of vagus stimulation. Thereafter there was a gradual reduction in the inhibitory effects but the oxygen consumption never returned to the normal rate, as long as the vagus stimulation was continued. The figures recorded in tables 2, 3, and 4 are calculated from the effects of the first five minute period of stimulation.

After-effects. In about 50 per cent of the experiments we noted a dis-

tinct acceleration of the rate of oxygen consumption after vagus stimulation was suspended, the rate exceeding the normal resting rate by a variable amount. The acceleration was limited to the first five minutes following stimulation and gradually tapered off to the normal resting rate. In spite of the intensity of this after-effect the acceleration never made up for the decreased oxygen consumption during the period of inhibition. In speculating on the cause of this augmented post-inhibitory phase in the chemical processes in the heart, it is conceivable that the inhibitory influence confines itself to the terminal step in the process of tissue oxidations inhibiting only the actual combination of oxygen with oxidizable substances. If this

TABLE 4

Difference in effect of one and of both vagi on O_2 consumption of turtles' auricles in cubic centimeters per gram per hour

Calculations based on five minute periods

TEMPERATURE	RESTING AURICLES	ONE VAGUS STIMULATION	PER CENT REDUCTION	BOTH VAGI STIMULATION	PER CENT REDUCTION	RESTING AFTER STIMULATION
°C.	*cc.*	*cc.*	*per cent*	*cc.*	*per cent*	*cc.*
24.0	0.151	0.112	25.8	0.103	31.8	0.164
23.0	0.263	0.161	38.7	0.154	41.4	0.23
23.0	0.173	0.138	20.2	0.132	23.7	0.208
24.0	0.278	0.218	21.6	0.194	30.2	0.276
24.0	0.279	0.24	14.0	0.206	26.16	0.281
24.0	0.278	0.211	24.1	0.194	30.2	0.278
27.0	0.275	0.23	16.36	0.212	23.0	0.30
24.0	0.210	0.185	12.0	0.1695	19.3	0.218
24.0	0.218	0.194	11.0	0.1775	18.57	0.234
22.0	0.140	0.125	10.71	0.102	27.14	0.144
22.0	0.1438	0.1135	21.1	0.106	26.3	0.140
22.0	0.204	0.1765	13.48	0.1575	22.8	0.194
22.5	0.243	0.159	34.5	0.134	45.0	0.229
22.5	0.229	0.1465	36.0	0.127	45.0	0.217
Average ..	0.2203	0.1721	21.87	0.1548	29.73	0.2223

were the case the reducing substances would continue to be formed, would pile up in the tissues during the inhibitory stage and would be oxidized rapidly when inhibition ceased. If this were so after long periods of inhibition one might expect a considerably greater rate of oxidation than with shorter periods; this however does not seem to be the rule. Another more probable physiological explanation would attribute the effect to the delayed action of sympathetic augmentor fibers which are known to run in the vagus trunk. Their predominant effect comes on later after vagus inhibition has subsided and would correspond in time with the accelerating effects we have noted. Such late augmenting effects on the rate of oxida-

tions of resting cardiac tissue have yet to be demonstrated for sympathetic nerves, but they are well recognized in other cardiac responses to sympathetic nerve stimulation.

A graph, figure 1, represents the actual course of an experiment, not included in the tables; it illustrates most of the features discussed above, it

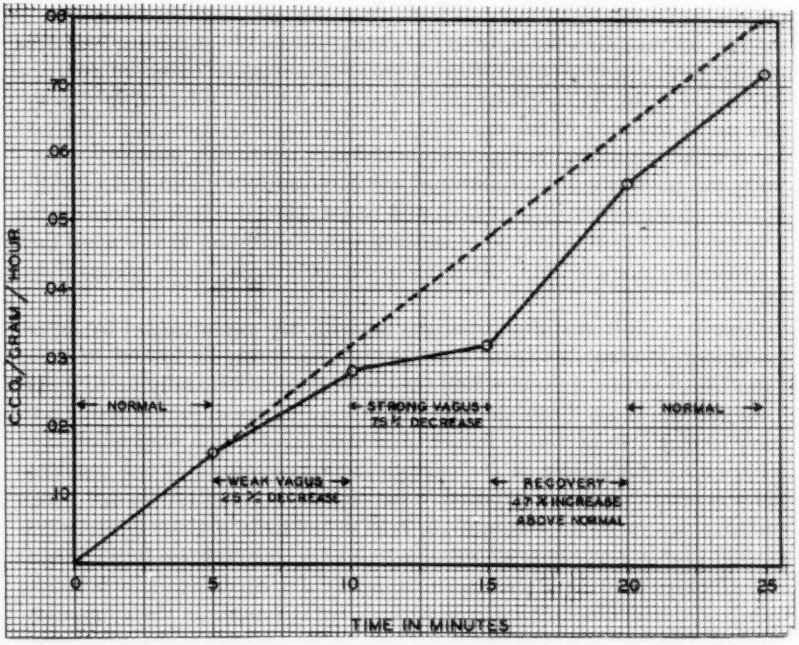

Fig. 1. A graph showing the rate of oxygen consumption by a resting preparation of turtle auricles. The first and last five minute periods show the normal resting rate. Strong vagus stimulation of one shows a greater depression during a five minute period than did weaker stimulation in the preceding equal interval. The post-stimulation augmentation of rate of utilization of oxygen is evident in this experiment. The broken line continues that representing resting oxygen consumption.

shows, e.g., the normal rate of oxygen consumption at the beginning of the experiment to be 0.16 cc. per gram-hour, that this was reduced 25 per cent by mild vagus stimulation and 75 per cent by stronger stimulation, that subsequent to vagus inhibition the oxidation rate for a five minute rest period became elevated to 0.22 cc. per gram-hour, an actual elevation of 47 per cent above the initial resting rate of oxygen consumption, and finally that the preparation settled down within five minutes to a condition

in which the oxygen was used at the same rate as during the initial rest period.

COMMENT. The experiments herewith recorded carry the conviction that vagus inhibition of the resting auricles of the turtle is accompanied by a decided change in the metabolism of this tissue as indicated by a decrease in the rate of oxygen utilization far below the level of the simple resting state. It is not to be inferred from these findings that this reduction of oxygen consumption is the essential characteristic of inhibition, for other studies to be reported subsequently indicate that similar reductions may be induced, e.g., by KCN without inhibition and without interfering with the functional integrity of the vagus nerves. Conversely there is evidence at hand which indicates that potassium chloride often may completely suppress the physiological responses of cardiac muscle without reducing the oxygen consumption, thus it may well be questioned whether these effects of potassium chloride can be considered to be inhibition in the sense that it causes physiological alterations identical with those due to vagus inhibition.

Recovery processes after muscular activity are accompanied by active oxidations; if these, or those dependent upon them, be looked upon as "anabolic," in the sense in which that term is conventionally used, it follows that the Gaskell concept that inhibition is an anabolic process is contrary to fact. Inhibition is rather to be associated with a suspension or at least with a diminution of normal metabolic processes. If one considers the so-called "positive variation," the electrical potential change which accompanies vagus inhibition (5, 6, 7, 8) in the light of these findings it is evident that it is a *relative* change due in reality to a decrease in the "negativity" which is an inevitable accompaniment of normal metabolic processes during activity which changes *pari passu* with changes in their intensity. Vagus inhibition thus by suppressing metabolic processes induces decreased negativity and therefore relative positivity.

SUMMARY

Inhibition of the turtle's auricles is accompanied by a reduction of oxygen consumption below the resting level. The stronger the vagus stimulation the greater is this depression of oxidations and simultaneous stimulation of both vagi is more effective than either vagus alone. The greatest reduction found amounted to 84 per cent of the resting oxygen utilization. These changes, herein recorded, probably do not constitute the essential feature of cardiac inhibition but are merely accompaniments. The findings are not compatible with the Gaskell anabolic theory of inhibition nor with the potassium theory; they throw new light on inhibitory "positive variation."

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CONDUCTION TIME IN THE AFFERENT TRACTS OF THE SPINAL CORD IN RELATION TO THE FLEXION REFLEX

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The flexion reflex is a defensive reaction of withdrawal of a limb from a noxious stimulus. It may be evoked in the decerebrate cat by an electric stimulus applied to an afferent nerve in the hind limb. Studies on the latency of this reflex were first reported by Sherrington (1906), contraction of the muscle being taken as the end point. Jolly (1911) measured reflex latency, using the electric response of the muscle; Forbes and Gregg (1915) made use of the electric response of motor nerve.

The above authors found the "reduced reflex time" to be approximately 4 σ. In other words, this amount of time was required for the impulse to pass through the spinal cord from the point of entrance at the posterior roots to the point of exit at the anterior roots. Eccles and Sherrington (1931) called attention to the fact that impulses on the afferent side of the flexion reflex arc travel at about 35 m. per second, which is slower than was previously assumed. Their estimate of the reduced reflex time was in fair agreement with those of Jolly, and Forbes and Gregg, the average being approximtely 3.6 σ.

In a report on the effect of ether anesthesia on afferent paths in the decerebrate cat, Forbes and Miller (1922) recorded the electric response of the medulla oblongata following stimulation of the sciatic nerve. Their results show an interval of about 8 σ from the peripheral stimulus to the appearance of action currents in the medulla. Forbes (1929) called attention to the implication of these time relations, viz., that the reflex discharge must be initiated before the arrival of sensory impulses in the cerebrum.

We undertook to make a more direct and complete comparison between the time of arrival of impulses in the medulla and the time of discharge of motor impulses in the flexion reflex, by making both measurements in the same animal.

METHOD. Cats were decebrated under ether anesthesia by the Sherrington guillotine method, at a level between the superior and inferior colliculi.

Afferent stimuli were applied to the popliteal nerve. The peroneal nerve

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was selected for the detection of the motor nerve impulses involved in the flexion reflex. To this end the sciatic nerve was cut near the knee and the above-named branches dissected apart. Stimulating electrodes (Forbes, Davis and Lambert, 1930) were applied to the popliteal nerve. The peroneal nerve was led into a moist chamber and placed on leading-off electrodes about 2 cm. apart, the nerve being crushed near the distal lead to render the response monophasic. The limb was immobilized by sectioning the nerves or tendons of all muscles acting on the hip or the knee.

To record the arrival of impulses at the medulla oblongata the posterior arch of the atlas and the underlying dura were removed. An electrode was inserted beneath the surface of the medulla just below the level of the gracile and cuneate nuclei. The circuit was completed through another electrode applied to the cut surface of the brain stem. The leading-off electrodes were usually of the Ag-AgCl type. In some experiments wicks impregnated with agar and saline were employed (cf. Forbes and Miller, 1922). Stimuli were delivered by means of a balanced induction coil similar to that described by Bishop (1926).

Two recording systems were used, one consisting of a Hindle galvanometer with a 130 mm. string and an amplifier. The original one-stage amplifier described by Forbes and Thacher (1920) was replaced by a two-stage condenser-coupled circuit using no. 224 and 227 tubes. The other recording system consisted of a Cambridge galvanometer with a short string (6 mm. long and less than 2μ in diameter), and a six-stage amplifier, described by Forbes, Davis and Emerson (1931). In the later experiments this six-stage amplifier was replaced by a four-stage condenser-coupled circuit using tubes no. 222 in the first and second stages, 102D in the third, and 247 in the fourth stage.

With the Hindle galvanometer the camera described by Forbes and Thacher (1920), using moving film, was employed. Single make and break shocks were delivered by means of a hand-operated key. With the short string galvanometer the camera described by Forbes, Davis and Emerson (1931), using a rotating mirror, was employed. To avoid fatigue or after-discharge resulting from immediately preceding stimuli, it was important that the stimulus be applied to the nerve after an adequate period of rest. This was achieved through the method described by Forbes, Davis and Emerson, consisting of an automatic shutter which opened during a single revolution of the mirror and which carried a key which short-circuited all but the single break shock delivered while the shutter was open.

The absence of lag in the short string galvanometer and the high speed of recording, 3 to 8 m. per second, made it possible to determine the latency with higher precision than was possible in the earlier researches (Forbes and Gregg, 1915; Forbes and Miller, 1922).

At the conclusion of each experiment the following measurements were

made: the length of the popliteal nerve from the stimulating electrode to the upper root of entrance to the cord, the spinal cord from this point to the active lead on the medulla, and the peroneal nerve from its roots of origin to the proximal lead.

RESULTS. The measurements of the nerves and spinal cords showed the following significant figures for the distances over which the various impulses were conducted:

Popliteal nerve (afferent)	13 to 17.5 cm.	average 15.5 cm.
Peroneal nerve (motor)	15 to 20 cm.	average 17.4 cm.
Spinal cord	23 to 32 cm.	average 29.4 cm.

Flexion reflex. The flexion reflex was recorded from 25 animals. Using the stimulus artefact (escape of current) to mark the time of stimulation, measurements were made of the gross reflex time to the appearance of the response in the motor nerve. A number of measurements thus made from individual observations in the case of each animal were averaged. In 14 out of the 25 animals thus studied the average was 8 σ. The remainder ranged from 6 σ to 9 σ. Allowing for the time of conduction in the peripheral nerve at the fairly well established velocities, we find that a gross reflex time of less than 7 σ would indicate a reduced reflex time so far below those deduced in previous researches that we believe they should be discarded as erroneous, probably due to the fortuitous excursion of the string before the true response. The average of the remaining measurements for the entire series is 8 σ. The average for those measurements made with the more rapid of the two recording systems is about 7.5 σ. If we accept the average velocities as measured by Eccles and Sherrington, 35 and 80 m. per second respectively for the afferent and motor nerves, we arrive at an average reduced reflex time of 1.4 σ. If we accept the highest velocities found by Eccles and Sherrington, viz., 40 and 97 m. per second respectively, we still arrive at an average reduced reflex time of approximately 2 σ, which is considerably less than the lowest value, 2.75 σ, which Eccles and Sherrington derived from their observations.

The question arises whether a difference in experimental conditions could have caused a real difference in reduced reflex time in our experiments from that which was obtained in theirs, or whether a systematic source of error enters into one or other of the two series of observations, which would explain the apparent difference. Their method of arriving at the reflex time was more indirect than ours, but it was so designed as to eliminate certain presumable sources of error. Their experiments also included an actual measure of conduction time in the peripheral nerves, which in our experiments can only be inferred from the length of nerve measured and the assumption of similar conduction velocities. Our method of recording revealed the most rapid motor impulses in the nerve, and some of these may

have been faster than those transmitted to the tibialis anticus muscle, which were the only ones involved in their procedure. Those of our experiments made with the short string (cf. fig. 2A) had the advantage of a more rapid recording system, using a string with less inertia.

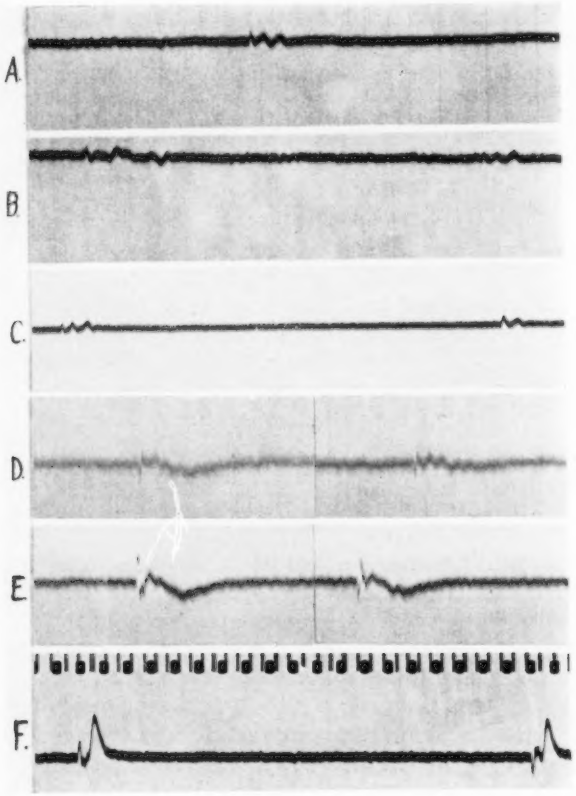

Fig. 1

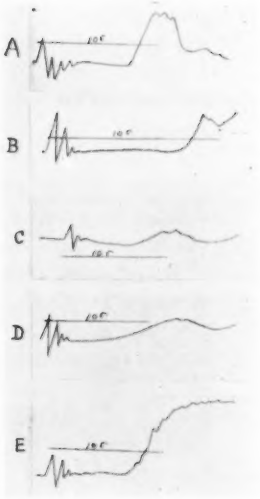

Fig. 2

Fig. 1. Electric responses in medulla compared with those of motor nerve involved in the flexion reflex. Records made with Hindle galvanometer (130 mm. string). A–E inclusive, response of medulla. A and C, AgCl electrodes; B, D and E, agar wick electrodes. F, flexion reflex. A shows a single shock, each of the others shows a pair of shocks, make and break. The time of stimulus is shown by the artefact preceding the response. Speed of film the same in all, shown by time marker (0.01 sec.) at top of F.

Fig. 2. Records made with short-string galvanometer and rotating mirror. A, flexion reflex. B–E, inclusive, response of medulla. Time of stimulus shown by beginning of oscillatory artefact, oscillations due to periodicity of string at high tension. Time is shown by 10 σ line on each record. Records retouched.

It is safe to assume that no change in experimental conditions other than a difference of temperature is likely to alter materially the conduction velocity in peripheral nerves. The observations of Eccles and Sherrington show that the reduced reflex time may in consequence of a previous stimulus be reduced to a value as low as 0.5 σ. If, therefore, the difference between our measurements and theirs as to gross reflex time is real and not merely a result of observational errors, it would be reasonable to suppose that the difference was in the reduced reflex time, rather than in the speed of peripheral conduction, and that some unknown difference in experimental conditions had in some way influenced the condition of the center.

Response in medulla. Examination of over 500 records from 25 animals showed the earliest electric response in the medulla oblongata usually between 6 σ and 7 σ after stimulation of the popliteal nerve. This signifies an average velocity of conduction amounting to about 65 m. per second for the entire distance from the point of stimulation to the medulla. The gradual onset of the excursion suggests a wide distribution of individual axon velocities, and nearly all the records show a second peak in the electric response (see fig. 1 A to D; in fig. 2 only the first part of the response is shown). This second peak usually occurred about 25 to 30 σ after the stimulus. If it signifies the arrival of a second group of afferent impulses their average velocity from the source of stimulus to the medulla would be about 16 m. per second. It is conceivable, however, that they represent cellular responses similar to those designated "intermediary potentials" by Gasser and Graham (1933).

Discussion. Measurements of conduction velocity in the spinal cord reported by Gasser and Graham (1933) since our experiments were completed, indicate a velocity of 80 m. per second through the region of the lumbar enlargement; throughout the remainder of the cord above this point they found evidence that the impulses were conducted at about 30 m. per second. It is interesting to note in comparison with their measurements that the earliest response in the medulla in our records indicates an average velocity of 65 m. per second for the entire distance, whereas the second peak of the electric response in our records occurs at a time representing an average velocity of conduction of about 16 m. per second.

If we should take the value, 35 or 40 m. per second, which Eccles and Sherrington reported for the velocity of afferent impulses in the flexion reflex, as obtaining also in the case of the afferent impulses to the medulla, it would imply a velocity of more than 100 m. per second in the spinal cord. In view of the observations of Gasser and Graham this appears unlikely. Heinbecker, O'Leary and Bishop (1933) found afferent fibers in the cat's saphenous nerve conducting at velocities of 60 to 80 m. per second (cf. also Erlanger and Gasser, 1924). It is probable that these more rapid impulses are in those neurones which extend without interruption to the medulla,

and give rise to the first electric response detected at that point. This would imply an average velocity of conduction in the cord of 50 m. per second, which is not far from the values found by Gasser and Graham. The slower impulses which evoke the flexion reflex would then contribute nothing to this first medulla response, but might be represented in the second peak of the disturbance observed there.

It is evident from our measurements that the stimulus which evokes the flexion reflex activates the anterior horn cells of the spinal cord at approximately the same time as, or even before, any impulses set up by the stimulus arrive in the medulla oblongata. In estimating the earliest possible time of arrival at the cortex, we must allow at least 0.4 σ for each synapse, the shortest known time for conduction through such a structure (Eccles and Sherrington, 1931), and at least 1 σ for conduction in the intervening axons. It appears then that impulses cannot reach the cortex in less than 8.0 σ after peripheral stimulation. This is at least 4 σ after the arrival of the peripheral impulses at the spinal cord.

Comparing this time with the reduced reflex time of 2 to 4 σ, it is evident that the reflex discharge in the motor neurones occurs before the sensory impulses can reach the cerebral cortex in the intact animal. It is generally agreed that the cortex is the seat of conscious recognition of sensory impulses. If this is correct, it is evident that such defensive mechanisms as the flexion reflex are effected before the animal can become aware of the exciting stimulus.

SUMMARY

The reflex time in the flexion reflex, measured by the appearance of the action current in the motor nerve, is compared with the time of arrival of the first electric response in the medulla oblongata, arising from the same stimulus applied to the popliteal nerve.

The measurements, made with a high speed recording system, taken in connection with peripheral conduction velocities measured by previous workers, appear to indicate a reduced reflex time of about 2 σ.

The first response in the medulla appears about 6.5 σ after the stimulus, and probably involves more rapidly conducting afferent fibers than those involved in the flexion reflex. The total response in the medulla suggests a fairly wide distribution of individual conduction velocities.

Even assuming the reduced reflex time for flexion to be as long as 4 σ, it is concluded that the motor neurones discharge in this defensive reflex before the first sensory impulses could reach the cortex in the intact animal.

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GLUCOSE AND NON-GLUCOSE PORTIONS OF "BLOOD SUGAR" IN THE HEPATIC AND PORTAL VEINS OF THE DECAPITATE CAT AT DIFFERENT SUGAR LEVELS

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Claude Bernard (1849) drew from his experiments the conclusion that in a dog whose diet contained neither sugar nor starch there was no sugar in portal blood. This conclusion has been shown to be erroneous for portal blood, even in fasting animals, has been found to contain sugar. Cori, Cori and Goltz (1923), using an abdominal window method in rabbits, found the difference in sugar content of blood from liver veins and neck veins to be approximately 28 mgm. per cent; and from liver vein and femoral artery to be approximately 23 mgm. per cent. Kotchneff (1928), using dogs with cannulae permanently inserted into various blood vessels, found some 20 mgm. more sugar in hepatic than in portal blood when the dogs were fasting.

In these experiments the animals were not under anesthesia at the time of observation, and it is possible that some disturbance in blood sugar might result from the manipulations involved in drawing blood. To obviate this difficulty and to study the possible differences in hepatic and portal blood over a wide range of concentrations of blood sugar, we have used cats decapitated under ether anesthesia.

The cats were without food (unless otherwise stated) 16 to 24 hours before decapitation. It was found more feasible not to draw blood simultaneously from the two veins, but to take it first from the hepatic, then as quickly as possible (1 to 3 minutes later) from the portal or a large tributary. When more than 3 samples from each vein were to be taken, less than 1 cc. was drawn each time, and the total reducing substance was determined on 0.2 cc. after precipitation with tungstate; the glucose content of a similar sample was obtained after precipitation with zinc salts. The difference between total reducing substance and glucose fraction gives the non-glucose portion of "blood sugar." With certain large cats determinations were made on full cubic centimeter samples, but the results were always perfectly comparable with those when the smaller quantity of blood was used.

We have determined the glucose fraction of blood in hepatic and portal veins over a range of over 300 to less than 75 mgm. per cent (125 samples). In the vast majority of cases there was significantly more glucose in hepatic than in portal blood; in the remainder there was no significant difference in the blood from these two veins. A typical result is shown in table 1 (cat 2).

It has been shown that although liver glycogen decreases during the time the "blood sugar" falls after decapitation (Olmsted and Coulthard, 1928; Evans, et al., 1931–1932), the increase in glycogen cannot be entirely accounted for by changes in blood sugar. Our present results show that while glycogen is being deposited in the liver there is either actually more glucose leaving the liver than entering it, or there is no significant change in glucose content as blood passes through the liver. Our results therefore provide further evidence that liver glycogen cannot be formed directly

TABLE 1

HOURS AFTER DECAPITATION	TOTAL REDUCING SUBSTANCE			GLUCOSE			NON-GLUCOSE FRACTION		
	Hepatic	Portal	Difference	Hepatic	Portal	Difference	Hepatic	Portal	Difference
½	305	262	43	268	241	27	37	21	16
2¼	260	239	21	230	222	8	30	17	13
3½	194	190	4	180	170	10	14	20	−6
4¾	178	166	12	143	135	8	35	31	4
Asphyxia	321	256	65	256	240	16	65	16	49

from glucose in the blood, otherwise one would expect the liver to remove sugar from the blood, certainly not to add glucose to it.

In order to be certain that the excess glucose in hepatic blood was not coming to the liver from arterial sources, in several cats ligatures were placed around the hepatic and gastroduodenal arteries, and cystic duct as well (see fig. 2, Evans, Murphy, and Young, 1932). Following this operation, the liver is supplied by blood from the portal vein only. We can confirm Evans, Murphy, and Young (1932) that even under these conditions the glycogen content of the liver increases after decapitation, e.g., ½ hour after decapitation glycogen was 1.29 per cent; 2½ hours, 1.23 per cent; 4¾ hours, 1.67 per cent; after asphyxia, 1.2 per cent.

The differences in glucose content of hepatic and portal blood were even more striking in these preparations with the hepatic artery tied than in those with arterial supply to the liver intact (table 2). It is perhaps significant that the least difference was observed in a cat which had been kept without food for 48 hours and whose liver, therefore, was presumably somewhat low in glycogen. It may also be noted that in a cat decapitated

TABLE 2

CAT	HOURS AFTER DECAPITATION	TOTAL REDUCING SUBSTANCE			GLUCOSE			NON-GLUCOSE FRACTION			REMARKS
		Hepatic	Portal	Difference	Hepatic	Portal	Difference	Hepatic	Portal	Difference	
1	½	272	291	61	240	187	53	32	24	8	
	4¼	233	157	76	195	115	80	38	42	−4	
	5	211	145	66	175	115	60	36	30	5	
	Asphyxia	379	235	144	339	196	143	40	39	1	
2	¼	259	224	35	226	198	28	33	26	7	
	2½	149	124	25	129	103	26	20	21	−1	
	3¾	129	100	29	108	81	27	21	19	2	
	Asphyxia	336	215	121	300	178	122	36	37	−1	
3	½	183	152	31	152	129	23	31	23	8	
	2½	127	103	24	106	84	22	21	19	2	
	3¾	123	95	28	100	72	28	23	23	0	
	Asphyxia	237	210	27		173			37		
4	½	255	225	30	230	200	30	25	25	0	5 1-gram samples of liver removed during course of experiment
	2½	170	155	15	155	140	15	15	15	0	
	4½	130	105	25	110	85	25	20	20	0	
	Asphyxia	350	200	150	325	180	145	25	20	5	
5	½	194	180	14	166	160	6	28	20	8	Stomach full of food. 1 cc. samples
	5½	97	89	8	83	65	18	14	24	−10	
	Asphyxia	280	250	30	263	234	29	17	16	1	
6	¼	191	191	0	166	162	4	25	29	−4	1 cc. samples
	4½	91	86	5	76	63	13	15	23	−8	
	Asphyxia	203	187	16	170	156	14	33	31	2	
7	¾	292	262	30	263	239	24	29	23	6	1 cc. samples
	4	157	135	22	132	109	23	25	24	1	
	Asphyxia	384	248	136	354	224	130	30	24	6	

during active digestion of fish meal there was also more glucose in hepatic than in portal blood.

With the exception of certain samples taken shortly after asphyxia,

when there was as much as 145 mgm. per cent more glucose in hepatic than in portal blood, there appears to be no correlation between the difference in glucose content of hepatic and portal blood, and the glucose level. Thus in cat 2, while the glucose in hepatic blood fell from 226 to 108 mgm. per cent, the difference in glucose content of hepatic and portal blood remained the same. This is also well shown in cats 3 and 7. This suggests that the difference is to be accounted for by the use of glucose in metabolism. The exceptions after asphyxia may possibly have been due to circulatory disturbances, since asphyxia was allowed to proceed until the heart beat was very feeble and the blood pressure practically zero before artificial respiration was restored.

The non-glucose portion varied between comparatively narrow limits, 65 to 15 mgm. per cent being the extremes. The average amount for hepatic and for portal blood was exactly the same, viz., 25 mgm. per cent. Immediately after decapitation and after asphyxia there was a slightly higher proportion of non-glucose "blood sugar" than at other times; this is undoubtedly related to the increase in the number of corpuscles thrown into circulation by the contraction of the spleen at these periods.

SUMMARY

In decapitate cats without food since the previous night the glucose portion of "blood sugar" is generally significantly higher in hepatic than in portal blood. The degree of difference is with few exceptions relatively constant over a considerable range of "blood sugar" levels, the average difference being 28 mgm. per cent when the liver is supplied by portal blood only. Since these determinations were made while glycogen was being deposited in the liver, it is most unlikely that liver glycogen can come directly from glucose in the blood.

The non-glucose fraction is fairly constant over a wide range of "blood sugar," being slightly higher immediately after decapitation and after asphyxia. There is no difference between hepatic and portal blood with respect to the non-glucose fraction.

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ABSENCE OF LIGHT AND THE REPRODUCTIVE CYCLE IN THE GUINEA PIG

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Previously reported observations on the normal reproductive behavior of the female guinea pig have revealed that during the period February 15 to May 15, inclusive, oestrous activity in this species is predominantly nocturnal, the frequency of oestrus between 6 p.m. and 6 a.m. being twice that between 6 a.m. and 6 p.m. (Young, Myers and Dempsey, 1933). In addition, the most probable time for oestrus occurrence was found to shift toward the late night hours as the days lengthened. Two problems suggested themselves immediately: first, the nature of the nocturnal influence, and secondly, the cause of the shift in the mean point of oestrus occurrence as the days lengthened.

A third problem developed more or less secondarily. It had been shown recently by Bissonnette (1932a, b) for such seasonal animals as the starling and the ferret and by Baker and Ranson (1932) for the vole that these species can be stimulated to partial reproductive activity out of season by simply increasing the light ration to which the animals are exposed. These observations had led to the suggestion that the sexual cycle in seasonal animals is controlled by the amount of light to which they are exposed, although Marshall (1932) and Baker and Ranson realized that such a mechanism could not account for the breeding of some animals in the autumn when the number of daylight hours is decreasing, or for reproductive activity in subterranean animals, cave animals, and animals which are active only at night. In view of these observations it became of interest to determine if light is in any way related to reproductive activity in a polyoestrous form. The experimental data bearing upon this latter problem and on the two former problems are given below.

The nature of the nocturnal influence. Our investigation of this aspect of the problem seemed best begun by observing the behavior of animals kept in a completely darkened room in which the visible changes from daylight to darkness and vice versa were not detectable. Such an environment was established by screening off a corner of a ventilated photographic darkroom. It was illuminated by a green light of low luminous intensity only at the time of the observations and feeding. Thirty adult females, each of which

had been observed in heat at least once, were placed in the darkroom and observed at two hour intervals day and night throughout three and four reproductive cycles during the period October 15 to December 15, 1933. The assumption of the copulatory posture was used as an indication of oestrus and data were recorded with respect to the time of day the animals came into heat. A control was provided by the observation of 90 animals maintained simultaneously under normal conditions.

The results suggested that the change from daylight to darkness is the factor which operates to bring the animals into heat during the night hours. To be sure, the first oestrous period following the removal of the animals to the darkroom occurred more frequently during the night than during the daylight hours (Dempsey, Young, Myers and Jennison, 1933). Subsequently, however, the behavior changed and the animals came into heat as frequently during the hours of darkness as during the hours of daylight

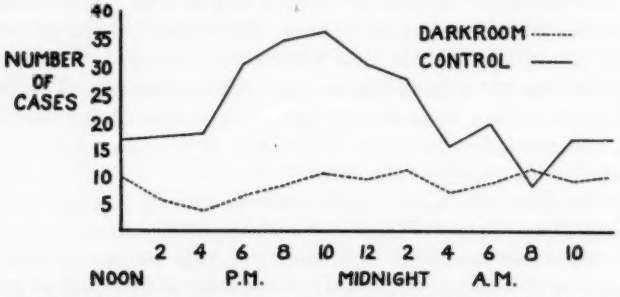

Fig. 1. Relationship between the occurrence of oestrus and the time of day in the darkroom and control groups.

(fig. 1). Support for the conclusion that the flattening of the curve during the latter part of the period of observation is not a matter of chance but represents a real effect of the changed environment comes from a statistical examination of the time of occurrence of the 94 oestrous periods involved. Application of Fisher's (1928) X^2 test for a straight line shows a value for P which lies between 0.7 and 0.8; a value showing a close, but not unreasonably close, agreement with the hypothesis tested since values of P above 0.05 are to be regarded as significant. This would indicate that the removal of the animals from the influence of daily alternation of daylight and darkness also removes them from the influence which is responsible for the nocturnal occurrence of oestrus.

The seasonal shift in the mean point of oestrus occurrence. This part of the problem involved a comparison of the data from 442 cases of oestrus observed during the period February 15 to May 15, 1933 with 271 cases observed during the period October 15 to December 15, 1933. If the shift

in the mean point of oestrus occurrence which occurred during the former period were attributable to the lengthening day, then a corresponding shift in the opposite direction should be detected during the second series of observations, October 15 to December 15, when the days are becoming shorter.

What has been interpreted as such a shift occurred. During the period October 15 to December 15, the occurrence of oestrus was predominantly nocturnal as it had been during the spring, 62.3 per cent of the cases occurring between 6 p.m. and 6 a.m. Nevertheless, a shift occurred in the mean point of oestrus occurrence from 11:38 p.m. in the spring to 10:12 p.m. in the fall. This shift of one hour sixteen minutes toward the early evening hours during a period when the days are shortening represents a difference which is 6.2 times its probable error. In addition, we have compared the extent of this shift with the change in the average time of sunset for the two periods. The latter was one hour forty-one minutes earlier during the October-December observations, a change which is of the same order of magnitude and in the same direction as the change in the mean point of oestrus occurrence.

The suggestion made previously that the occurrence of oestrus is associated very closely with the approach of darkness rather than with some more subtle nocturnal influence would seem, therefore, to receive two-fold support from the data reported above: 1. The occurrence of oestrus ceases to be essentially nocturnal when animals are removed from an environment in which the alternation of daylight and darkness is detectable. 2. The shift in the time of the probable occurrence of oestrus from a season when the days are longer to a season when the days are shorter is comparable with and in the same direction as the shift in the time of sunset.

The reproductive processes in the absence of light. For investigation of this last problem it was simply necessary to extend our observations on the animals confined in the darkroom to include the duration of oestrus and the length of the reproductive cycle, to examination of the vaginal smears, and to a determination of fertility. In no one of the latter respects, however, was any abnormality noted in the events of the reproductive cycle. Only in the change in the time of the probable occurrence of oestrus indicated by the leveling of the frequency curve was the behavior of these animals different.

Data were obtained for the length of 60 entire reproductive cycles and 94 oestrous periods and are compared with corresponding data from the control group (table 1). In neither case is the difference significant.

This conclusion is open to the criticism that the animals which were observed were sexually mature at the time the experiment was started, therefore, that they had been exposed to light during a part of their lives. It is certainly conceivable that any effect which might occur would remain un-

noticed until after the expiration of a longer period of time than that over which the experiment extended. For this reason, six pregnant females were placed in the darkroom, where their young were delivered. Of these, four males and three females were raised in the darkroom and were not exposed to light until after the conclusion of the experiment.

In the case of the females, the vaginal closure membranes were observed to open cyclically, and two of the animals were found in heat. This shows clearly that oestrus can occur even if an animal has never been exposed to light. For the males also, the mating reactions appeared to be normal. Females in oestrus were placed with these animals and normal mating followed by impregnation occurred.

As a final check on the normality of the reproductive cycles of these animals which had been confined in the darkroom during three and four dioestrous cycles, seven females were mated for the purpose of determining their fertility. Of these, five were found to have been impreg-

TABLE 1

Length of the reproductive cycle and of the oestrous period in the darkroom and control groups

	REPRODUCTIVE CYCLE	OESTROUS PERIOD
	hours	*hours*
Darkroom group	393.3 ± 2.33	7.97 ± 0.19
Control group	383.3 ± 1.29	8.21 ± 0.07

nated, so that here again the behavior of the animals was not altered by their removal from a normally lighted environment.

The above cited evidence for the normal reproductive capacity of guinea pigs confined and actually born in a darkroom forces us to the conclusion that neither differences in the daily light ration nor a light ration itself is important for the reproductive processes of at least one polyoestrous species.

That this result is not inconsistent with conditions in the starling, ferret and vole as revealed by Bissonnette and by Baker and Ranson is indicated in a recent paper by Hill and Parkes (1934). These authors confined ferrets in light-proof cages during the time when the daily period of light was increasing and showed that the animals so treated came into heat at the same time as those exposed to light. Their conclusion is, that while an increased light ration may have the effect of hastening the appearance of the breeding season, it is not necessary for the occurrence of the breeding season when it is normally due. Since this has been shown, it is not surprising that the reproductive processes in the guinea pig are perfectly normal in the absence of light. They too would seem to be regulated by a

basic rhythm which is superficially modifiable by certain external factors,—in this species, the approach of darkness. But whether the approach of darkness or an increasing light ration is involved, these external factors do not seem to be essential for the existence or maintenance of the rhythm. If they are eliminated, the rhythm remains.

CONCLUSIONS

1. The confinement of 30 female guinea pigs in a completely darkened room was followed after the first oestrous period by a loss of the tendency to come into heat at night rather than during the daylight hours. In addition, the mean point of oestrus occurrence during the period October 15 to December 15, 1933 was one hour sixteen minutes earlier than during the period February 15 to May 15, 1933. This shift is 6.2 times its probable error and is of the same order of magnitude and in the same direction as the change in the time of sunset.

From these data it has been concluded that the change from daylight to darkness is the factor which is responsible for the more frequent occurrence of oestrus between 6 p.m. and 6 a.m. than between 6 a.m. and 6 p.m.

2. Observations on the duration of oestrus and the length of the reproductive cycle, examination of the vaginal smears, and a determination of fertility revealed that in these respects the reproductive cycles of animals excluded from any influence of light was normal. From this it is concluded that exposure to light is not necessary for the existence or maintenance of the reproductive rhythm in at least one polyoestrous species, the guinea pig.

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THE CREATINE CONTENT AND THE WEIGHT OF THE VENTRICLES IN EXPERIMENTAL HYPERTHYROIDISM AND AFTER THYROPARATHYROIDECTOMY

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Recent investigations have shown that experimentally produced thyrotoxicosis causes several alterations in the chemical makeup of the myocardium. However, no reports concerning the concentration of creatine are available. The fact that the compound phosphocreatine is decreased (3, 5) does not indicate whether creatine itself is actually lost from the myocardium, or whether the resynthesis of the compound is interfered with in the thyrotoxic state. Abelin and Spichtin (1) found that creatine was actually lost from the skeletal muscles and livers of animals fed thyroid substance, but they did not study the hearts of their animals.

The present investigation was undertaken to determine the effect of both hyper- and hypo-thyroidism upon the creatine concentration as well as the total amount of creatine in the ventricles of experimental animals.

METHOD. The animals used in this work were all young adult male rats from our own stock. They were fed a standard mixed diet throughout the experiment. Crystalline thyroxin "Roche" was administered subcutaneously to the 54 animals used in the thyrotoxicosis experiments. Thyroparathyroidectomy was done from 29 to 174 days (average 69 days) previous to sacrificing the 26 animals that comprised the hypothyroid group. Thirty-one normal rats were used as controls. Each animal was killed by a blow on the head followed by bleeding from the severed vessels of the neck. The ventricles of the hearts were cut away by careful section through the atrioventricular ring. They were then opened, blotted between filter papers to remove all free blood, weighed, and analyzed for total creatinine by the method of Rose, Helmer and Chanutin (6). The results of the analyses are reported in terms of creatine.

RESULTS. Table 1 summarizes the findings. The theoretical average weight of the ventricles at the beginning of the experiment was calculated for each group of experimental animals by multiplying the average initial body weight for that group by the normal ventricular wt./body wt. ratio. Likewise, the theoretical average total amount of creatine in the ventricles at the beginning of the experiment was calculated by multiplying the

above figure by the normal creatine concentration. The rest of the data found in the table is self-explanatory.

Chronic hyperthyroidism. The thyroxinized animals are presented in two groups. Those in the first group are designated as chronic hyperthyroid rats, since they were given thyroxin in divided doses over periods of time ranging from 7 to 33 days, with an average of 21 days. The total amount given to each of these animals varied from 1.5 to 5.0 mgm., and averaged 3.65 mgm. The single doses were in most instances 0.5 mgm., although a few 0.7 and 0.9 mgm. doses were given. These animals were killed from 1 to 4 days after the last of the series of injections.

TABLE 1

Data on normal, hyperthyroid and hypothyroid rats

The number of cases is indicated in parenthesis at the head of each column.

	NORMALS (31)		HYPERTHYROID, CHRONIC (46)		HYPERTHYROID, ACUTE (8)		HYPOTHYROID (26)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Initial body weight, grams			254	36	233	15	285	45
Initial ventricular weight, mgm. (calculated)			736.6		675.7		826.5	
Final body weight, grams	215	35	240	32	224	16	288	61
Final ventricular weight, mgm. (observed)	620.4	101.1	865.3	100.7	781.5	51.0	829.6	140.4
Ventricular weight / body weight ratio (final)	2.90	0.20	3.64	0.39	3.49	0.13	2.92	0.30
Creatine concentration in ventricles, mgm. per cent.	198	14	155	17	121	5	183	13
Initial total creatine in ventricles, mgm. (calculated)			1.46		1.34		1.64	
Final total creatine in ventricles, mgm. (observed)	1.22	0.18	1.33	0.17	0.95	0.08	1.51	0.20
Mgm. of creatine in ventricles per 100 grams body weight (final)	0.57	0.04	0.56	0.05	0.42	0.03	0.53	0.06

The concentration of creatine in the hearts of this group averaged 21.7 per cent below normal. At the same time the ventricles had hypertrophied to the point where the mean ventricular wt./body wt. ratio was 3.64, or 25.5 per cent above normal. The calculated actual gain in ventricular weight was 17.5 per cent. The calculated actual loss of creatine from the heart was only 9 per cent. Thus, although the concentration of creatine was markedly reduced in these ventricles, the concurrent cardiac hypertrophy approximately made up the difference, keeping the total amount of creatine in the ventricles fairly constant.

Acute hyperthyroidism. The experiments on the second group of hyperthyroid rats showed that creatine can be caused to leave the heart in considerable quantity by thyroxinization. These animals received massive single doses (5–10 mgm.) of thyroxin. They were killed on the fourth day after injection. Single doses of 5 mgm. were apparently as effective in regard to creatine loss as were doses up to 10 mgm. The creatine concentration for this group averaged 38.9 per cent below normal. Within the space of 4 days the ventricles of this group had apparently gained in actual weight 15.7 per cent of their calculated initial weights. The calculated average loss of creatine from the hearts was 29.1 per cent.

Hypothyroidism. The results found for the thyroparathyroidectomized rats were less pronounced. There was no change in ventricular weight. The concentration of creatine in the ventricles was only 7.6 per cent lower than normal; a difference which is significant, however, as proven by Fisher's formula (4) in which the value found for t was 4.0. This slight lowering of creatine concentration might be due to a rise in the water content. However, alterations in water content would not change the total amount of creatine in these hearts. The observed total ventricular creatine was 7.9 per cent lower than the calculated initial value. The average ventricular creatine per unit of body weight (when based upon either initial or final body weights) is significantly lower than normal as shown by the fact that the value of t in this comparison is greater than 2.9.

It is rather surprising that both hyper- and hypothyroidism should affect the creatine of the heart in the same direction. The hyperthyroid heart is characterized by its strong, rapid contractions, and its increased muscle mass, while the heart in hypothyroidism is characteristically weak, slow, sluggish, and dilated. They seem to have opposite characteristics except in three particulars. First, it is known that heart failure is not an uncommon clinical finding in both hyperthyroidism and hypothyroidism. In this regard, it may be pointed out that in a study made on human material, it was found that most of the hearts which had failed had a creatine concentration definitely lower than normal (2). Second, the glycogen of cardiac muscle is markedly decreased in hyperthyroidsm, and slightly so in hypothyroidism. Third, the creatine in the heart apparently suffers the same alterations as glycogen, in these two conditions.

CONCLUSIONS

Thyroxin, whether administered in divided doses over a period of time, or in a single large dose, causes an actual increase in ventricular muscle mass (in spite of a lowering of general body weight). There is a decrease in creatine concentration in the ventricles, and an actual loss of creatine from the heart.

Thyroparathyroidectomy, while producing no change in the weight of the ventricles, does cause a slight but significant loss of creatine from the heart.

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THE EFFECT OF IRON WITH AND WITHOUT OTHER ELEMENTS UPON THE PRODUCTION OF POLYCYTHEMIA[1]

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Observations are on record which show that several inorganic elements may produce polycythemia when administered in suitable doses. These are As, Mn, P, Ge, Co, and Cu. Among these Co has recently attracted the most attention in this connection. Waltner (1) and Waltner and Waltner (2) observed a polycythemia and increase in hemoglobin above normal when Co was fed. Mascherpa (3) also observed increases in both Hb and erythrocytes after the oral administration of Co to dogs. Myers, Beard and Barnes (4) reported that 1 per cent Co added to their stock diet produced striking increases in Hb and erythrocytes.

Lewis and co-workers (5) (6), Stare and Elvehjem (7) and Brand and Stucky (8) have also observed a polycythemia when Co was fed to rats maintained upon a basal milk diet supplemented with Fe and Cu. Lewis and co-workers believe that the Co polycythemia produced by the Waltners and ourselves (4) was due to the presence of Cu in the diets used. We did not observe a polycythemia in our rats on the stock diet, but did so when Co was added. This, of course, does not exclude the possibility that the Cu in this stock diet was responsible for the Co polycythemia, as suggested by Lewis.

Since it is impossible to prepare a diet free from Cu and to prevent some storage of this element in the body of the rat before the experimental periods are begun Lewis' view of the necessity of Cu for the production of Co polycythemia cannot be proved or disproved experimentally at the present time. In most nutritional anemia studies the Cu content of the diet is held as low as possible, then the effect of *added* Cu in very small doses is tested. Our purpose is, therefore, to use normal adult rats and to note the effect of additional Cu (over that present in the body of the rat and milk) upon the production of the Co polycythemia.

[1] A preliminary report of these studies was made before the Division of Biological Chemistry, at the 87th Meeting of the American Chemical Society, March 28, 1934, at St. Petersburg, Fla.

Young adult rats were used and Hb and red cell counts were made weekly on a few drops of blood obtained by clipping the end of the tail, using the technique previously described (9). Twenty-five hundredths milligram Fe with milk was fed daily to each animal, with the exception of the group on the stock diet, for a period of 6 weeks. Our first study was conducted with 76 animals, and the second study was made some time later with an additional 23 animals, with identical results. Hence table 1 contains the average values of Hb and erythrocytes for all animals studied.

TABLE 1

Effect of inorganic elements and vitamin D with Fe upon production of polycythemia. Average values

NO. OF RATS	BODY WEIGHT		HB. PER 100 CC.		R.B.C. PER C.MM.		DURATION OF EXPERIMENT		DIET
	Before	After	Before	After	Before	After	Hb.	R.B.C.	
	grams	*grams*	*grams*	*grams*	*millions*	*millions*	*weeks*	*weeks*	
13	153	207	12.0	14.5	8.72	8.93	6	6	Stock
12	152	186	13.4	14.0	8.67	9.70	6	6	Milk + 0.25 mgm. Fe alone
8	134	176	10.0	11.8	8.02	8.91	6	6	Milk + 0.25 mgm. Fe + 0.05 to 0.8 mgm. V
12	136	174	11.3	13.0	8.05	9.75	6	6	Milk + 0.25 mgm. Fe + 0.15 to 0.4 mgm. Zn
8	130	179	10.5	13.4	7.42	9.07	6	6	Milk + 0.25 mgm. Fe + 0.075 to 0.2 mgm. Cu
9	183	203	13.5	15.5	9.02	9.26	6	6	Milk + 0.25 mgm. Fe + 0.2 mgm. Cu + vitamin D*
19	159	190	11.9	14.3	8.89	*11.47*	6	6	Milk + 0.25 mgm. Fe + 0.15 to 0.3 mgm. Co
9	178	194	14.6	*18.6*	9.48	*11.40*	6	6	Milk + 0.25 mgm. Fe + 0.3 mgm. Co + 0.05 mgm. Cu
9	171	187	13.6	*19.3*	9.20	*10.75*	6	6	Milk + 0.25 mgm. Fe + 0.3 mgm. Co + vitamin D*

* 1 drop of "SMACO" vitamin D concentrate added daily to the milk.

RESULTS. No polycythemia occurred in the animals fed the stock diet,[2] milk-Fe diet, and V, Zn, and Cu (with and without vitamin D) additions to the milk-Fe diet. The first evidence of polycythemia occurred in the group of 19 animals fed the milk-Fe diet supplemented with 0.15 to 0.30 mgm. Co. When Cu was added to the milk-Fe-Co diet, no greater polycythemia occurred than that obtained with this diet alone, but the final Hb value was 18.6 grams as contrasted to 14.3 in this group. In the last

[2] Sherman's diet B (⅔ whole wheat flour and ⅓ whole milk powder together with $CaCO_3$ and NaCl, each to the extent of 1 per cent of the weight of the wheat) with fresh lettuce 3 times per week.

group, vitamin D addition (1 drop of cod liver oil concentrate daily) to the milk-Fe-Co diet did not produce any greater polycythemia than Co alone, but again the final Hb value was 19.3 grams as compared to 14.3 grams.

Discussion. In our previous work (4) 0.5 to 1 per cent of V in the stock diet produced a decided polycythemia. In the present studies no polycythemia occurred when 0.05 to 0.80 mgm. V was added to the milk-Fe diet while 0.1 mgm. Zn added to the milk-Fe diet in the Cleveland studies resulted in a final erythrocyte count of 10.3 m.c.mm. and in the present study of 9.75 m.c.mm. In our recent study (10) of the effect of ultraviolet light and irradiated milk, with Fe, upon blood regeneration in nutritional anemia, we observed a polycythemia in 10 animals fed Fe alone and in 22 on Fe and irradiation. These results were not due to either Cu or Co but simply to an overstimulation of the blood-forming organs by the iron-light therapy which had formerly been withheld from the animals. We emphasized in our original publications (9) (11) the variations which may be encountered in the experimental animals in nutritional anemia studies. Recently Drabkin and Fitz-Hugh (12) have found differences in the blood picture of mature rats of two separate colonies. They pointed out that these differences in the rats themselves may be among the factors responsible for the discrepant results reported in the literature on nutritional anemia of the rat. Therefore, it seems reasonable to conclude that variations in recovery from nutritional anemia and the production of polycythemia are due to metabolic functions which may vary in different animals under the different experimental conditions.

Among the investigators who have produced polycythemia with Co, only Lewis and co-workers have stated that Cu was necessary for the production of this Co polycythemia. A careful study of their published evidence (13) shows only 4 rats fed on Fe and Co. We do not believe that these data furnished sufficient evidence for the view that Fe and Co will not produce a polycythemia. Contrasted with this we show in this paper 19 animals on Fe and Co, and 9 on Fe with Co and vitamin D (this vitamin being ineffective in the production of polycythemia in these experiments) making a total of 28 animals in which polycythemia was produced without the assistance of *added* Cu. It should again be emphasized that our studies do not prove or disprove Lewis' view of the necessity of Cu for the production of Co polycythemia for the reasons mentioned above, but they do show that added Cu is not necessary in this connection.

SUMMARY

Young adult rats have been fed for 6 weeks on a whole milk diet with 0.25 mgm. Fe added daily. V, Zn, Cu, Co and vitamin D were each added separately and in combination to this diet, and the increases in Hb and erythrocytes above normal were noted. The results obtained were as follows:

1. Twenty-five hundredths milligram Fe; 0.05 to 0.80 mgm. V; 0.15 to 0.40 mgm. Zn; 0.075 to 0.20 mgm. Cu and 0.20 mgm. Cu plus vitamin D did not produce a polycythemia.

2. Fifteen hundreths to 0.30 mgm. Co produced polycythemia with no increase in Hb above normal. Thirty hundredths milligram Co plus 0.05 mgm. Cu gave the same degree of polycythemia as Co alone but resulted in a greater increase in Hb.

3. Thirty hundredths milligram Co plus vitamin D resulted in moderate increases in both Hb and erythrocytes above normal.

4. The production of polycythemia is a metabolic function characteristic of the animal itself.

5. Cu, in amounts above that present in the body of the rat and milk, is not necessary for the production of the Co polycythemia.

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THE SOURCE OF OESTRIN IN THE PREGNANT MARE

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Data presented in previous articles have led us to adopt the view that the large amount of oestrin excreted in the urine of the pregnant mare is of fetal placental origin. The evidence upon which this view is based may be summarized as follows: The maternal ovaries in this species during the last third of pregnancy are fibrous structures rarely containing any large follicles (Cole, Howell and Hart, 1931). There is a correlation between the remarkable development of the fetal gonads and the oestrin content of the maternal urine. The highest concentration of oestrin in the urine is reached at about the time maximum development of the fetal gonads is attained and further the regression of the fetal gonads late in intra-uterine life is coincident with a drop in the oestrin content of the mare urine (Cole et al., 1933). We will explain why we are unable to accept the concept that the fetal gonads are secreting the oestrin. In assaying various maternal and fetal tissues of the horse for oestrin greater concentrations were found in the mare kidney, endometrium and fetal placenta than in either the maternal or fetal gonads (Catchpole and Cole, 1934). The concentration in the fetal gonads was comparable to the concentration in other body tissues of the fetus. Usually the hormones have been found most concentrated in the glands secreting them. The kidney has not been given serious consideration as the source. Its high concentration in this tissue is attributed to the fact that the hormone is being excreted by this organ. We consider the data on the hormone concentration in the mare urine preceding parturition and in dam and foal urine following parturition most decisive in regard to the source. Whereas oestrin has been found invariably in mare urine preceding parturition and in first voided foal urine, by 24 hours after parturition it cannot be detected in the urine of either the mother or her offspring (Catchpole and Cole, 1934). If the fetal gonads were secreting oestrin one might expect to find it in the foal urine for several days at least, as no marked morphological change occurs in the gonads at this time. The sudden disappearance of oestrin from the urine of both mother and foal following parturition suggests that the secreting organ had been suddenly ablated. The fetal placenta is ablated within a few hours after the expulsion of the fetus and we believe it to be the organ involved in the secretion of oestrin in the latter

part of pregnancy in the mare. It appeared desirable, however, to obtain more conclusive evidence on this point because there are many who object to the hypothesis that the same hormone may be secreted in more than one organ. Consequently we have ovariectomized one mare at about the 200th day of pregnancy.[1] The ovaries were removed with an ecraseur through a lateral abdominal incision on the right side and were found to be fibrous structures characteristic of this stage of pregnancy. A small area of the germinal epithelium investing the left ovary was sheared off and crushed in the chain of the ecraseur at the time of severance. It was necessary to remove the left ovary inside the peritoneal cavity because it could not be brought to the surface at the point of the incision in the right flank. The mare survived the operation, gave birth to a normal foal 147 days after the ovariectomy and lactated normally for 20 days after parturition at which time she was sacrificed. Evidence that there was no regeneration of the germinal epithelium sheared from the left ovary is given by the fact that the mare did not come into heat at the normal 9 day period following parturition. On post-mortem examination no evidence was found of ovarian tissue. There was some distention of the oviducts at the point of severance and on the left side the severance occurred so as to leave what appeared to be a small free end of the tube which was distended with fluid. To positively identify this tissue it was sectioned, stained and examined under the microscope when it was readily recognized as oviduct.

Urine samples were taken twice prior to the operation and at frequent intervals thereafter until 2 days post partum. The concentration of oestrin in the urine of this animal (mare C10) is compared in figure 1 with that of three normal mares at corresponding times preceding parturition. The oestrin content of the urine was determined with spayed rats by halving the dose until the minimal amount was found which would give a vaginal smear of nucleated epithelial cells, cornified cells, or both, with the absence of leucocytes in three out of four animals. This amount was considered as one rat unit. It will be observed (fig. 1) that there is considerable variation in the oestrin content of the same individual over relatively short periods. This is no doubt partly due to the fact that the concentration was determined by halving the dose (the testing of intermediate levels would have reduced this apparent variation) and also to the fact that we determined the oestrin contained in a small quantity of urine rather than from a twenty-four hour sample. With the methods used there is considerable individual variation at the same stage of pregnancy. For example, at 60 days prior to parturition the oestrin was less concentrated in the urine of

[1] A number of similar operations have been performed on the human female (Waldstein, 1929; Probstner, 1931, and others) but all do not agree that the question of ovarian participation in the secretion of oestrin during pregnancy has been conclusively settled.

mare C6 than in that of mare C10 while mare C8 was comparable to mare C10. On the other hand the urine of mare C3 shows a higher concentration during the greater part of the period reaching at two tests the high level of 33,000 rat units per liter.

Figure 1 shows a rather rapid and marked fall of the oestrin in the urine of mare C10 a few days after the operation. This was followed by a rise and during the last 60 days preceding foaling the oestrin content was comparable to that of the normal mares. We have no explanation for the temporary post-operative decline. Probstner (1931) found a similar decline

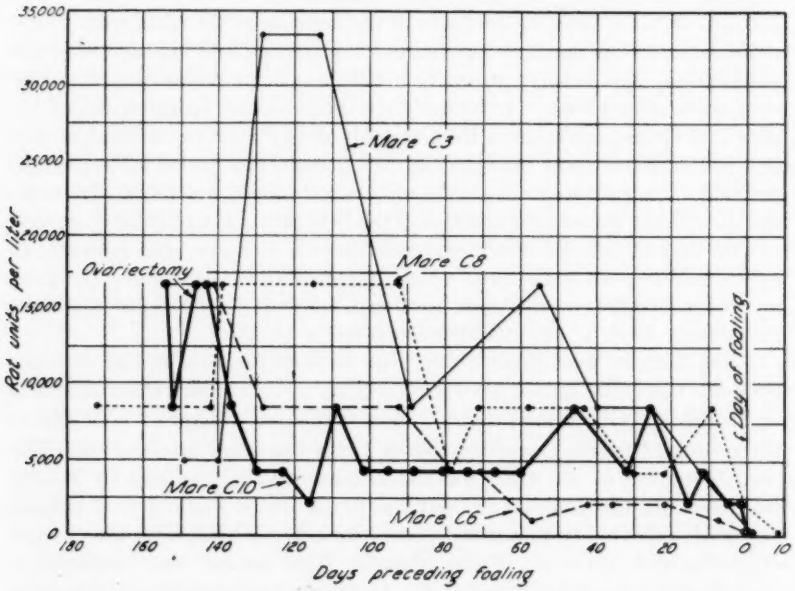

Fig. 1. The comparison of the oestrin concentration in the urine of an ovariectomized mare (mare C10) with that of three normal mares during the last 150 days of pregnancy.

in one human case following ovarian extirpation. On the day of foaling the urine of mare and foal contained small amounts of oestrin (250 rat units per liter) and two days later it was absent from the urine of both, which condition is normal for the species.

Despite certain limitations to our data we present the following convincing evidence that the maternal ovary does not appreciably contribute to the production of oestrin during the last third of pregnancy. Catchpole and Cole (1934) presented data on the oestrin content of horse tissues at periods of gestation corresponding to the time of ovariectomy of mare C10. The

parts in which oestrin was most concentrated (endometrium and mare kidney) contained about 7 rat units per gram whereas for most tissues assayed more than a gram of fresh tissue equivalent was required to represent a rat unit. The maximum amount of oestrin that the body of mare C10 weighing 800 pounds could have contained at the time of ovariectomy was $2\frac{1}{2}$ million rat units, (calculated on the basis of the most concentrated tissue) and it is likely that 300,000 rat units is nearer to the actual amount. Kemp and Pedersen-Bjergaard (1933) have shown that a considerable amount of oestrin is excreted in the feces of the pregnant mare in addition to that excreted in the urine. The average oestrin content of mare C10 urine following the operation was about 4,000 rat units per liter. The total urine excreted over one 24 hour period was found to be $15\frac{1}{2}$ liters. Thus, about 9 million rat units were excreted after ovariectomy in the urine alone. This was several times the amount that could have been present in the body of the mare at the time of the operation with the most generous estimation. Thus there is indubitable evidence that oestrin was secreted after the removal of the ovaries. Further, the close agreement of the oestrin level of the urine of the ovariectomized mare with that of the normal mares leads us to the conclusion that the maternal ovary does not contribute to the production of oestrin during the last third of pregnancy.

SUMMARY

A mare was ovariectomized at about the 200th day of pregnancy and the oestrin content of the urine was determined at frequent intervals for 150 days after the operation. Pregnancy proceeded normally in the absence of the ovaries. The mare gave birth to a normal foal and lactated normally. There was an initial drop in the oestrin level in the urine a few days after operation with a subsequent rise. During the last 60 days of pregnancy the oestrin content of the urine was comparable to that of normal mares. With the aid of previously published data it was shown that oestrin was produced after the operation and the conclusion is drawn that the maternal ovary is not concerned to an appreciable extent in the production of oestrin during the last third of pregnancy.

Other tissues which may be involved in the production of this hormone are the fetal gonads, fetal placenta or endometrium. We present evidence in this paper and summarize data from other work which we feel justifies the conclusion that this substance is secreted by the fetal placenta.

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A METHOD FOR EXPLANTATION OF THE KIDNEY

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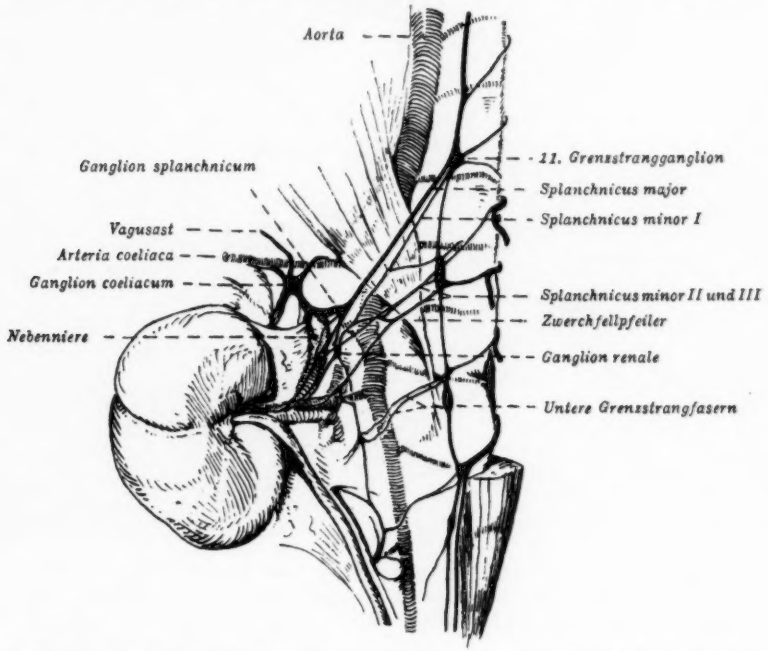
A method for obtaining samples of blood at will from the renal vein of the living, unanesthetized animal is required for the accurate determination of facts regarding the physiology of the kidney. Many studies of renal circulation, metabolism, and function are based upon observations of preparations so temporary and so far from normal in function that their validity is open to question. This communication presents a method of permanent surgical explantation of the kidney, described by Rhoads (1) in a preliminary publication. After healing has taken place the renal vein may be punctured and blood withdrawn as often as desired and in any amount. The method employed was suggested by the procedure for exteriorizing the spleen described by Barcroft (2). A somewhat more complicated procedure than that of Barcroft was required for experiments on the kidney since access to the renal vessels was desired. A method for simple explantation of the kidney has been described independently by Entz and Huggins (3). The technic used made access to the renal vessels difficult, if not impossible, and was not suitable for our purposes.

TECHNIC. Young, healthy female dogs, preferably weighing more than 15 kgm., are selected. Under morphine and ether anesthesia an oblique skin incision about 2.0 cm. below and parallel to the left costal margin is made. The muscle layers are split in the direction of their fibers and the peritoneum entered, exposing the kidney. The organ is gently freed in situ from the perirenal fat, and the renal vein and artery followed to their origins. The kidney is next brought outside the muscles laterally and anteriorly by swinging it in an arc with the pedicle as the radius and the origins of the vessels the center. This method avoids undue traction on the pedicle and leaves the kidney supported by the muscle layers, which have fallen together loosely beneath it. See plate 1, fig. 1. If the muscles have been properly split, no sutures will be required. It is of the utmost importance that ample space for the pedicle be left. Furthermore, every effort is made to avoid disturbing the nerve supply which is closely attached to the vessels. Study of text figure 1 will show how explantation without interference with nerve supply is possible. The kidney is supplied with nerve through the celiac and suprarenal plexuses. All the nerves to the

organ are found in the renal pedicle closely approximated to the vessels. No nerve fibers should be broken when the kidney is freed from the perirenal fat. All the nerves on the pedicle are left undisturbed when the organ is mobilized and brought to its new position outside the muscle layer.

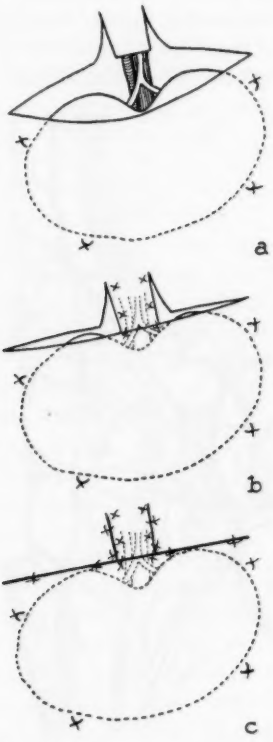
The kidney is next tipped posteriorly and held in this position by bringing the posterior skin flap over it tautly, covering the organ to the hilus. This posterior rotation of the kidney throws the renal vein into a prominent position at an acute angle to the animal's flank. The position is main-



Text fig. 1. The renal nerve supply in the dog. A. Hirt, Ztschr. f. d. ges. Anat. **78:** 261, 1926.

tained by suturing the posterior skin flap to the muscle layer snugly over each pole of the kidney. See text figure 2 a. The anterior flap is next incised at right angles to the original line of incision giving a short, rectangular flap attached anteriorly and just long enough to cover the renal vein and to meet the posterior flap. See text figure 2 a and 2 b. The rectangular piece is then sutured to the muscle snugly around the renal vein. Tension enough to interfere with venous return must be carefully avoided. This leaves the vein covered by an inverted gutter of skin. All that remains is to approximate the remaining free edges of the anterior to

the posterior flap and the anterior to the rectangular flap. This is accomplished by interrupted silk sutures. Text figure 2 c and plate 1, figure 2. Great care is required to obliterate any dead space which may fill with serum. A very thick absorbent cotton dressing is applied and fastened



Text fig. 2a. Diagrammatic representation of the operation. Compare with plate 1, figure 1. The posterior flap of skin has been brought over the kidney. The anterior flap has been incised to form the short piece used to cover the pedicle.

Text fig. 2b. The short rectangular piece of the anterior flap has been sewed over the pedicle to the muscle layer and its edge approximated to the posterior flap.

Text fig. 2c. Skin closure of anterior to posterior flap and of the small rectangular piece of the anterior flap covering the pedicle to the remainder of that flap has been completed.

firmly in place by many overlapping strips of adhesive tape. The dressing is kept in place for one week and then removed. At this time all skin sutures may be taken out except those immobilizing the poles of the kidney and the renal pedicle. These are removed at the end of the second week,

when it should be possible to feel the renal vein distinctly and to obtain blood from it at will. A certain amount of subcutaneous edema remains around the pedicle for a few weeks and gradually disappears. If the deep sutures have been properly placed a firm scar involving the muscle layer

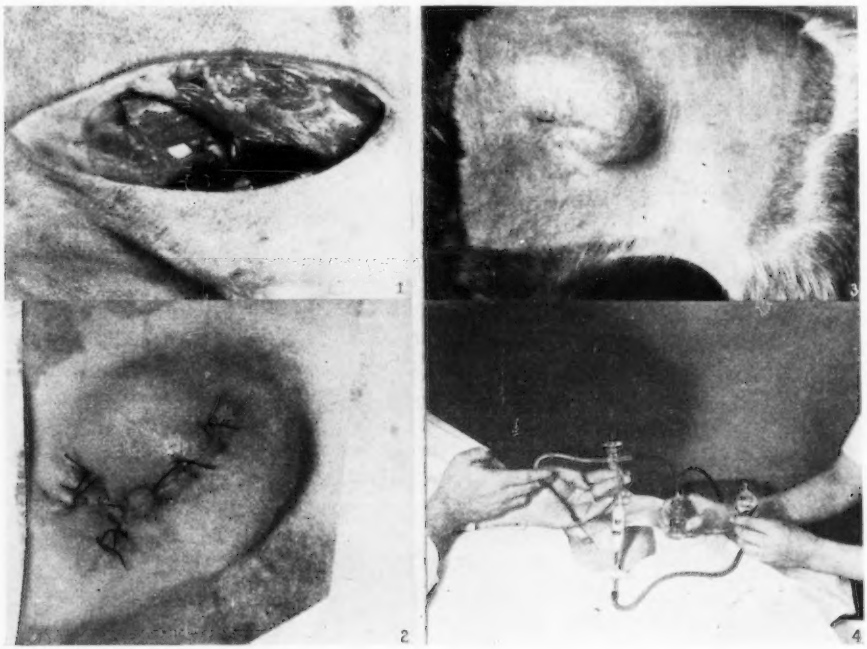


Plate 1, fig. 1. The skin and subcutaneous tissue have been incised, the muscle layers split and the peritoneal cavity entered. The kidney has been freed of perirenal fat and brought outside the muscle layer. The pedicle may be seen with the nerve supply in place.

Plate 1, fig. 2. The organ in place and skin closure complete. Some rotation of the kidney in its subcutaneous position has taken place.

Plate 2, fig. 3. The explanted kidney after healing has taken place. The pedicle is firmly fixed between the anterior scars.

Plate 2, fig. 4. Method of obtaining blood from the renal vein. No anesthesia is required. The blood may be collected under mercury or in an open bottle as desired.

remains on either side of the pedicle. This holds the pedicle relatively immobile and allows easy access to the renal vein. Plate 2, figure 3.

The presence of the explanted organ apparently causes no discomfort to the animal. After a time, the shape of the kidney becomes altered by the pressure of the surrounding tissues. The remaining kidney may be removed if analyses of the urine secreted by the explanted organ are desired.

In obtaining blood from the renal vein certain precautions must be observed. Plate 2, figure 4. Owing to the position of the kidney and pedicle the vessel must be entered very nearly at a right angle. The needle must be ground with a very short, sharp, deeply curved bevel. When the needle is properly used there is rarely danger of entering the renal artery.

Eighteen such preparations have been under observation for two years. They have been used repeatedly in experimentation and the non-explanted kidney has been removed without perceptible harmful results. Determinations of the effects of the operative procedures on renal function will be published elsewhere.

SUMMARY

A method for permanent surgical explantation of the dog kidney to a subcutaneous position in the flank is described. The method of fixing the organ in position is such that samples of blood may be obtained at will from the renal veins of living, unanesthetized animals.

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THE FUNCTIONAL EFFECT OF EXPLANTING ONE KIDNEY AND REMOVING THE OTHER

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The animals used in these experiments were dogs in which one kidney was explanted by the technique of Rhoads (1931, 1934), and the other kidney was subsequently removed.

The dogs received the stock ration used in the animal house. It was a mixed diet, to which raw meat was added every other day. The average protein content of the daily diet was about 4 grams per kilo body weight. At the beginning of the work we did not recognize the considerable extent to which the urea clearance in dogs can rise and fall with the protein content of the diet. Consequently no especial attention was paid to the constancy of the composition of the stock diet, or to the amounts consumed by the individual animals. In part the fluctuations of clearance values noted in some of the animals are perhaps of dietary origin. Later when the diet was controlled more carefully, clearance values were more constant.

Technique used for urea clearance determination. The urea clearance test, described by Møller, McIntosh, and Van Slyke (1928) for use with human subjects, was used as the test for kidney function, with slight modifications in technique to make the test applicable to the animals. Each urea clearance was done after a fasting period of 24 hours. Before the experiment 12.5 cc. of 0.3 per cent sodium chloride solution per kilo was given by stomach tube in order to obtain a good flow of urine.

The urine was collected by catheterization with soft rubber catheters. The bladder was washed twice with 30 cc. portions of sterile 0.9 per cent sodium chloride solution. The two washings were collected together as a sample separate from the undiluted urine. The exact time was noted when the catheter was removed at the end of the second washing. Undiluted urine and washings were analyzed separately for urea by the manometric urease method (Van Slyke, 1927; Peters and Van Slyke, 1932, p. 361). The volume of undiluted urine represented in the washings was calculated by multiplying the volume of the washings by the factor,

$$\frac{\text{urea concentration of washings}}{\text{urea concentration of undiluted urine}}.$$

The total volume of urine output during the period was calculated by adding, to the volume of undiluted urine obtained, the volume of urine represented in the washings. The concentration of urea in the blood was determined by urea analyses of arterial or jugular blood drawn at approximately the midpoint of the time of each period.

In our dog clearances the augmentation limit could not be fixed with any precision, but appeared to be above 0.2 cc. of urine per minute. In order to exclude urine volume as a factor in influencing the clearance, we therefore endeavored to assure volumes above 0.2 cc. per minute by administering 100 to 300 cc. of 0.3 per cent saline solution before each experiment. When the volume per minute fell below 0.2 cc. the urine was not used for clearance calculations. The clearances were all calculated by the formula for the "maximum clearance" per square meter of body surface:

$$\text{Urea clearance} = \frac{UV}{BA}$$

U = urea concentration in urine, B = urea concentration in blood, V = urine volume in cubic centimeters per minute, A = surface area of body in square meters.

The surface areas of the dogs were calculated by the formula of Cowgill and Drabkin (1927), which as published by them is

$$A \text{ (in cm}^2\text{)} = 2.27\ W^{0.70} \times \frac{L}{3\sqrt{W}} = 2.27\ W^{0.367}\ L$$

A = surface area in square centimeters; L = length of dog in centimeters from nose to anus; W = weight in grams. The formula was, however, for our use, more convenient when expressed in terms of square meters, kilograms, and meters. It is also convenient to put both the W factors under one exponent. Thus altered, the formula is:

$$A \text{ (in m}^2\text{)} = 0.2864\ W^{0.367}\ L$$

where W is weight in kilograms, and L is body length in meters, from tip of nose to root of tail.

Results. In figure 1 the maximum urea clearance, $\frac{UV}{B}$, is plotted against surface area in square meters for normal dogs, dogs with one normal kidney and one explanted kidney, and dogs with only one explanted kidney, respectively. The curves obtained show a proportionality between height of urea clearance and body surface, such as had previously been found for human subjects by McIntosh, Møller, and Van Slyke (1928). Urea clearances were therefore calculated per square meter of body surface by the formula $\frac{UV}{BA}$, A representing the surface area in square meters.

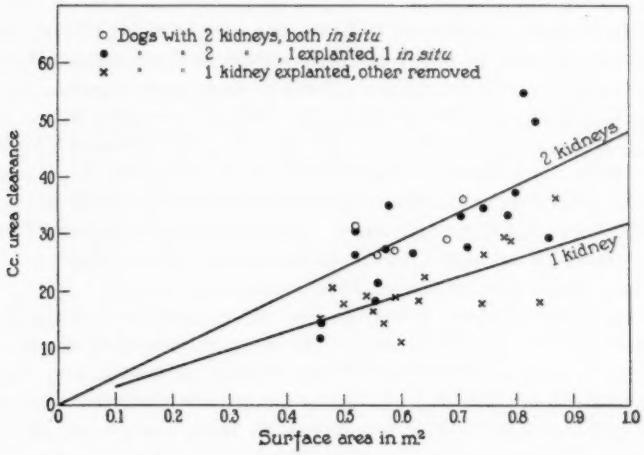

Fig. 1. The relation of urea clearance, $\frac{UV}{B}$, to surface area in normal dogs, in dogs with one kidney explanted and the other *in situ*, and in dogs with one kidney explanted and the other removed. Each point represents the average obtained with one dog.

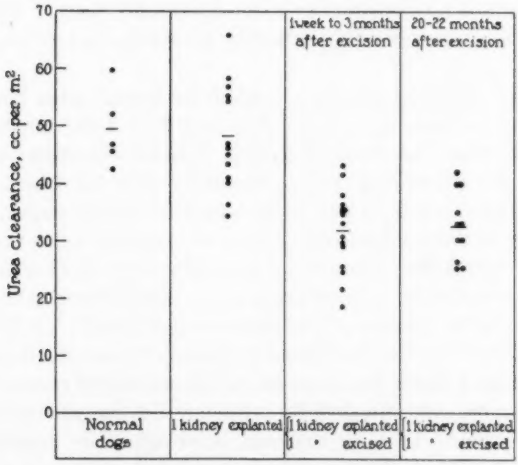

Fig. 2. The effect of explantation of one kidney and of subsequent removal of other kidney on urea clearance, $\frac{UV}{BA}$. Each circle represents the average clearance obtained with one dog.

A dash, —, indicates average of all dogs in the group.

The average urea clearance obtained by us from 26 observations on the five normal dogs was 49.8 cc. of blood per square meter of body surface. This does not differ greatly from the average urea clearance obtained for normal dogs by other authors. Twenty clearances reported by Ralli, Brown, and Pariente (1931) from data in which V exceeded 0.2 cc., give an average urea clearance of 57.7 cc. per square meter. Data of Jolliffe and Smith (1931) from observations in which V exceeded 0.2 cc. yield a mean clearance of 55.3 from 49 observations on 10 dogs on a mixed diet containing 55 to 110 grams of protein daily. Summerville, Hanzal and Goldblatt (1932) found an average of 53.1 obtained from estimations on 17 dogs. Holman's (1933) average was 45.5 for his normal control.

The explantation of one kidney had no effect on the urea clearance, when the other kidney was left *in situ*. The average clearance obtained from 101 observations on 13 dogs with one kidney explanted and the other kidney undisturbed was 48.1 per square meter body surface. This approximates our average of 49.8 for dogs with both kidneys in the normal position. (See data in fig. 2.) The clearance level before and after explantation of one kidney in the individual animals was also unaffected.

After removal of the unexplanted kidney the urea clearance decreased. From 48.1 before unilateral nephrectomy the average clearance fell to 31.9. This value was obtained from 247 observations on 16 dogs from one week to three months after excision of the unexplanted kidney. The results are shown in figure 2, and are also indicated by comparison of the two curves in figure 1.

The average clearance after removal of the unexplanted kidney sank to 64 per cent of its previous value. The fact that it did not fall to 50 per cent indicates that the residual kidney became somewhat more active, and thereby partly compensated for the removal of the other. Blood flow and oxygen consumption values, to be detailed in a later paper, also showed more activity in the residual kidney after the removal of its companion.

Clearance values were followed for over 20 months in 11 dogs. The final clearance values showed in most cases no marked difference from those obtained shortly after excision of the unexplanted kidney. It is certain that there was no tendency for the explanted kidneys to lose functional efficiency (fig. 2). Figure 3 shows the more usual behavior after removal of the unexplanted kidney, with no definite tendency for the clearance to change in either direction. In some animals, however, there appeared to be a gradual increase in the clearance, such as might have been expected if the remaining, explanted, kidney had been undergoing hypertrophy. It was not usually pronounced enough to exceed definitely the range of spontaneous variations. The most definite of such increases that we observed is shown in figure 4.

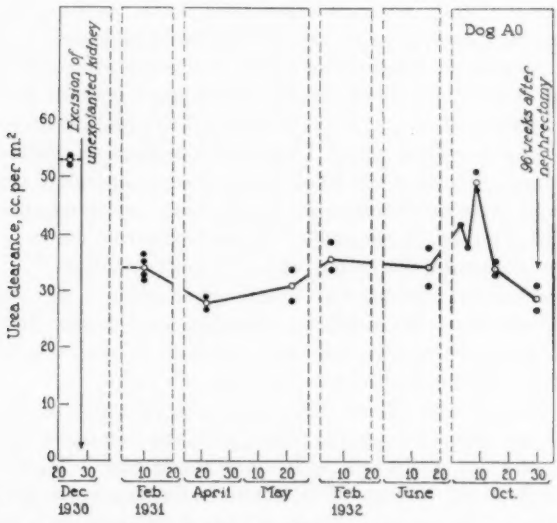

Fig. 3. Urea clearances observed during 96 weeks after removal of one kidney from a dog, the remaining kidney having been already explanted. The results show, as in most of our animals, a relatively constant function of the remaining, explanted, kidney.

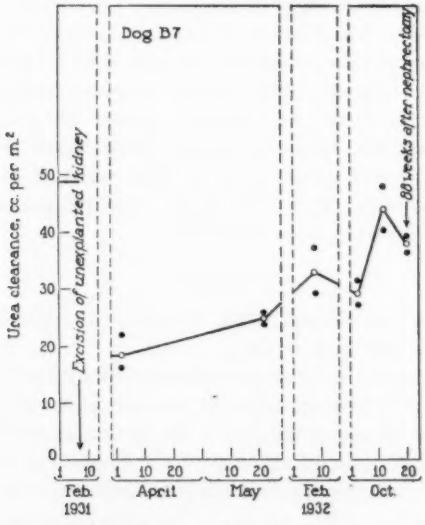

Fig. 4. Urea clearance observed during 88 weeks after removal of one kidney from a dog, the remaining kidney having already been explanted. In this case there was a slow increase in function of the remaining, explanted kidney.

Discussion. Other experimenters who have performed unilateral nephrectomy on animals have noted, besides the initial increase in functional activity, somewhat more regular evidence of increase either in size or function of the remaining kidney during subsequent months following the operation. Addis, Myers, and Oliver (1924) performed unilateral nephrectomies on rabbits. They noted, 15 to 33 days after the operations, that the average urea clearance was 63 per cent of its pre-operative value. Subsequently the average clearance gradually rose, and 4 months after the nephrectomy attained 79 per cent of the pre-operative value. There was also some anatomical hypertrophy of the remaining kidney.

Such hypertrophy, measured by weighing the kidneys, has been demonstrated in young rats by MacKay, MacKay, and Addis (1932). They found that the hypertrophy was most marked in the rats which were youngest when the one kidney was removed.

Karsner, Hanzal, and Moore (1934) followed the clearance in 4 mature dogs before and after unilateral nephrectomy, the remaining kidney being left *in situ*, and not explanted, as in our experiments. Their results are somewhat ambiguous, because of variations in the clearances obtained with individual dogs. In general, however, the data indicate a decrease in urea clearance after the operation, and a more or less complete restoration of it to normal value in the course of six months. There was also some hypertrophy, indicated by increase in weight of the residual kidney by from 3 to 27 per cent over the weight of the kidney first removed.

In man it appears that removal of one kidney may usually be followed by eventual rise of the urea clearance to a level well within the range observed in normal subjects. The writers (unpublished) have for several years observed such a case, with almost exactly 100 per cent of average normal urea clearance. Ellis and Weiss (1933) report 9 cases, free from complications, in which sooner or later after removal of one kidney the urea clearance was found within the range, 70 to 127 per cent of mean normal, covered by entirely normal subjects.

SUMMARY

Explantation of one kidney, while the other was left *in situ*, caused no change in the urea clearance of dogs.

Subsequent removal of the non-explanted kidney caused the urea clearance to decrease to an average value 64 per cent of the original.

The fact that the clearance fell only to 64, instead of 50 per cent, indicates an increase in the functional activity of the remaining kidney. This increase was apparent during the first weeks following the unilateral nephrectomy.

Subsequently the clearance of the single, explanted kidney, during periods of observation extending up to two years, either remained constant or showed a tendency to increase.

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RELATIONSHIPS BETWEEN UREA EXCRETION, RENAL BLOOD FLOW, RENAL OXYGEN CONSUMPTION, AND DIURESIS. THE MECHANISM OF UREA EXCRETION[1]

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In a normal man the volume of blood cleared of urea per minute by the kidneys averages about 75 cc. In a given subject the physiological variation attributable to functional elasticity of the normal kidney may be as much as ± 30 per cent of the mean urea clearance.[2] (Möller, McIntosh, and Van Slyke, 1928.) In a dog the variation produced by dietary and other changes may be much greater.

Rise or fall of blood urea in a given subject does not alter his urea clearance; excretion rate rises parallel with blood urea concentration, so that a minute's excretion continues to represent the urea content of the same volume of blood. This was demonstrated by urea administration to normal men by Addis and Drury (1923), and to both normal and nephritic men by Möller, McIntosh and Van Slyke (1928). Drury (1923) found it possible to increase the blood urea of rabbits 50-fold without affecting the clearance. Data in the present paper show that the clearance values for dogs are not affected by 10-fold increase in the blood urea.

When the secreting elements of the kidney are inactivated or destroyed

[1] Preliminary reports of part of the experimental results have been published in abstract by Rhoads, Van Slyke, Hiller and Alving (1931), and by Van Slyke, Rhoads, Hiller, and Alving (1931).

[2] The term "urea clearance" is used, in the sense defined by Møller, McIntosh, and Van Slyke (1928), to indicate the number of cubic centimeters of blood, the urea content of which is excreted in one minute (see also Peters and Van Slyke, *Quantitative Clinical Chemistry*, Vol. i, p. 345). The normal variation of ±30 per cent found in the clearance in man is considerably exceeded in the dog; Jolliffe and Smith (1931) have found that dogs on high protein diets may show clearances twice as great as on cracker-meal diets.

When the urine volume falls below a certain limit, about 2 cc. per minute in men, defined by Austin, Stillman, and Van Slyke (1921) as the augmentation limit, the blood urea clearance ceases to be independent of the urine volume, and begins to decrease with decreasing urine output. The present paper, however, is concerned only with conditions in which the urine volume is above the augmentation limit, so that diuresis does not act as a factor influencing urea excretion.

by renal disease, on the other hand, the urea clearance may sink to 5 per cent of its normal value. At this point uremia either intervenes or is imminent (Van Slyke, Stillman, Møller, *et al.*, 1930).

The primary objects of search in the present work were two, viz., 1, the factors which are responsible for the relative constancy of the urea clearance in the face of great variations of blood urea concentration, and 2, which of these factors suffers change when the clearance does alter.

The factors which we have studied with these objects have been the rate of renal blood flow and the proportion of urea removed from the blood during its flow through the kidney. These two factors necessarily determine the clearance, which can be calculated as their product. Decrease in the clearance may be brought about by decrease in either factor or in both.

We have also sought to find whether, in the excretion of urea and of water at greatly varied rates, the excretory work of the kidney involves related variations of renal blood flow or oxygen consumption.

EXPERIMENTAL. *General plan of procedure.* The animals were dogs in which one kidney had been explanted by the technique of Rhoads (1931, 1934), in order to facilitate the drawing of blood from the renal vein. In some of the animals the other kidney was left *in situ*, but in most cases it was removed, in order to make more definite the relationship between urea excretion and the observed removal of urea from renal blood.

The usual diet contained 4 grams of protein per kilo daily; two-thirds of the protein was in the form of raw meat.

Each experiment was performed after a fasting period of 24 hours. Before each experiment 12.5 cc. of 0.3 per cent NaCl solution per kilo body weight were given by stomach tube in order to obtain a flow of urine well above the augmentation limit.

Each experiment as a rule included 3 or 4 successive one-hour periods of 40 to 60 minutes each. The dog was catheterized at the beginning of the experiment and at the end of each period. At approximately the middle of each excretion period a blood sample was drawn from the renal vein, and immediately afterwards another was drawn from the femoral artery. The time interval between the two samples was usually 2 to 5 minutes, but was sometimes as long as 10 minutes. The urea and oxygen contents of the blood from the artery and the renal vein were plotted against time on a large scale on coördinate paper. For comparison of arterial and venous contents, either the arterial content was found by graphic interpolation at the moment the renal blood was drawn, or both arterial and renal contents were found by interpolation for the moment at the mid-point of the period of urine collection. For calculations of blood flow and renal oxygen consumption, blood values interpolated to the middle of each urine collection period were used. For calculation in tables 3 and 4 of the proportion of urea removed from the blood by the kidneys, however, arterial blood urea

values were interpolated to the moments at which the renal venous blood was drawn: this procedure permitted making use of the preliminary and terminal pair of analyses in each day's experiment, as well as the analyses near the middle of each collection period. The rates of change of arterial blood urea concentrations were sufficiently regular to make it appear that the interpolations introduced no significant errors. In a few instances where excretion of urea momentarily stopped, a precipitous rise occurred in the urea content of the renal vein blood. This phenomenon, which will be discussed later, either occurred to a striking degree or not at all, so that there appeared to be no difficulty in detecting it. When it occurred the data were not used for blood flow calculations. The nature of the curves from which the interpolations were made is indicated by figures 1, 2 and 3.

Collection and preservation of blood samples. The blood samples were collected over mercury, without contact with air, in oxalate-containing tubes, by the method of Austin *et al.* (1922) slightly modified as described on page 54 of Peters and Van Slyke (1932). As soon as the blood was mixed with the oxalate the tube was completely immersed in icewater, and was kept at 0°C. until the oxygen determination, which was performed in less than an hour. It was found that no measurable change in oxygen content occurred in the cooled blood during the period intervening between the drawing and the analysis.

Methods of blood urea and oxygen analyses. In order to obtain reliable figures for the amounts of urea and oxygen removed from the blood by the kidneys an unusual degree of analytical accuracy was required, because the amounts removed were calculated from differences between the arterial and renal blood. Errors of 1 per cent in determinations of oxygen and urea would produce errors usually in the neighborhood of 5 and 10 per cent respectively in the calculated proportions removed. It was necessary also to analyze small blood samples, because the number of bleedings made during each experiment precluded drawing large single portions. The technique outlined below, with 0.5 cc. blood samples, was designed to meet these requirements.

Oxygen content was determined in 0.5 cc. samples by the method of Van Slyke and Neill (1924) as described on p. 323–324 of Peters and Van Slyke (1932). Each manometric reading, both before and after absorption of O_2 with hyposulfite, was repeated two or more times, with the gas at 0.5 cc. volume, until the operator felt certain that the reading was accurate within 0.3 mm. The duplicate readings were made as quickly as they could be with accuracy, in order to avoid slight errors from physical reabsorption of O_2 or N_2. Before each reading the meniscus in the chamber was lowered 1 or 2 cm. and then brought back to the 0.5 cc. mark, so that each reading constituted a check on the accuracy with which the 0.5 cc. volume of the gas was set. A frosted light was placed 0.5 meter behind the extraction

chamber to give a sharp definition of the meniscus, and other precautions, described on p. 243–4 of Peters and Van Slyke (1932), were taken to obtain accuracy in setting the meniscus at the 0.5 cc. mark.

Oxygen capacity was determined by analyzing in the same manner blood which had been saturated with atmospheric air at room temperatures of 20°–24°C. In calculating the O_2 bound as HbO_2, 0.5 volume per cent was subtracted, as physically dissolved O_2, from the total oxygen content of the blood (Van Slyke and Neill 1924).

Urea was determined by the gasometric urease method (Van Slyke, 1927; Peters and Van Slyke, 1932, p. 373) in 0.5 cc. samples of whole blood. The technique described by Van Slyke (1927) for 1 cc. samples was followed, except that half as great a volume of each reagent was used, and the total volume of solution extracted in the chamber was 3.5 instead of 7 cc. The manometer readings were made with the gas at 0.5 cc. volume when the blood urea concentrations were within ordinary range, but at 2.0 cc. volume when the blood urea was increased several-fold by urea injection. Factors for calculating the urea were computed from the CO_2 factors of Van Slyke and Sendroy (1927).

Accuracy of the blood analyses. Duplicate *oxygen* analyses usually agreed within less than 0.1 volume per cent, or 1 part in 200.

In *urea* determinations, the percentage accuracy varied with the amount of urea present. When the urea nitrogen content was only 8 to 10 mgm. per 100 cc., the average deviations between duplicates were of the order of 1 part per 100. As the blood urea content increased the relative accuracy of its determination became greater; with blood urea nitrogen in the neighborhood of 100 mgm. per 100 cc. differences between duplicates were usually below 1 part in 300, and in some experiments averaged as low as 1 part in 500.

When duplicates disagreed by more than the usual limit, there was ordinarily sufficient blood to make a third analysis, so that the average error in the values used is less then than the average difference between duplicates. It was almost invariably found, if the first duplicate analyses did not agree, that the higher result, for either oxygen or urea, was the correct one. We believe that in such cases the low result was usually caused by delivering the blood sample too rapidly from the 0.5 cc. pipette, so that drainage was not complete. The manometer readings on a given sample could be checked with such accuracy that errors exceeding the usual limits mentioned above could not be attributed to the gas measurements.

Collection and analysis of urine. Catheterization, collection of urine, and washing of the bladder were carried out as described in the previous paper (Rhoads, Alving, Hiller and Van Slyke, 1934). The urea concentrations in the urine and washings were determined by the gasometric urease method (Van Slyke, 1927; Peters and Van Slyke, 1932, p. 361).

Calculation of urea clearance. The urea clearance was calculated as described in the preceding paper (Rhoads, Alving, Hiller and Van Slyke, 1934). For comparison of results from animals of different size, the clearances were calculated per square meter of body surface, the surface being estimated by the length-weight formula of Cowgill and Drabkin (1927).

Calculation of renal blood flow. The rate of renal blood flow was calculated by comparison of the amount of urea removed by the kidneys from each cubic centimeter of blood with the rate of urea output per minute in the urine. E.g., if the rate of output is 25 mgm. of urea N per minute, and the difference between arterial and venous blood urea N is 0.1 mgm. per cubic centimeter, it is obvious that 250 cc. of blood per minute must flow through the kidneys in order to give off the observed 25 mgm. of urea N. The general equation is:

$$F = \frac{E}{A - R}$$

F = flow of renal blood in terms of cubic centimeters per minute.
E = excretion rate of urea N in milligrams per minute.
$E = U \times V$, where U is the urea N content of the urine in milligrams per cubic centimeter, and V is the urine volume in cubic centimeters per minute.
A = urea N content of arterial blood in milligrams per cubic centimeter.
R = urea N content of the renal vein in milligrams per cubic centimeter.
The values of A and R for calculation of renal blood flow were estimated by interpolation for the moment in the middle of each period of urine collection (e.g., see tables 1 and 2).

The procedure used, with renal blood taken by the technique previously described, provides, so far as the writers are aware, the first data concerning the renal blood-flow rate obtained without operation, anesthesia, or instrumental interference with the renal circulation.

The procedure suffers from a thoretical source of inaccuracy in that the rate of urea excretion, E, at the moment when the renal blood is drawn is estimated as the average of the urea excretion rate observed during the period of an hour. When the kidneys are functioning normally, however, the rate of urea removal from the blood, and hence presumably of urea excretion, appears to be fairly constant during such a period. This constancy is evident from comparison of the arterial and renal blood urea curves in the upper left hand corner of figure 1.

When both kidneys are present, and the urea removal is determined only by analysis of the renal blood from the one explanted kidney, the assumption is made, in calculating the total flow through both kidneys, that both function at the same rate with respect to blood flow and urea removal. This assumption may not be exact in all cases, although it appears to be approxi-

mately so, both from our data and those of Verney (1929). To eliminate error from functional differences between the two kidneys, however, we have based our conclusions, concerning relationship between renal blood flow and urea clearance, on experiments in which only the explanted kidney was present.

In calculating the blood flow per gram of kidney in the first four two-kidney dogs of table 5 the weight of the kidney was estimated from the size of the animal, as indicated in the footnote to the table. From each of the other four dogs the unexplanted kidney was later removed, and it was assumed that the weight of the two kidneys in each animal was twice the weight of the removed kidney.

In the cases of the one-kidney dogs in table 6 it was assumed that the weight of the explanted kidney left in each animal was the same as the weight of the kidney which was removed. This procedure makes no allowance for the possibility that removal of one kidney may be followed by hypertrophy of the other. Whether hypertrophy occurred in our animals we can not say, because they are all still alive. Such hypertrophy has been found by MacKay (1932) to follow unilateral nephrectomy in young rats, and to be greater the younger the rats are when nephrectomized. The question appears to be of no importance for the intrepretation of our results, so long as it is clear that the data for blood flow and O_2 consumption given per gram of kidney refer to the original weight of the organ, without such change as may have occurred after removal of its companion.

Calculation of renal oxygen consumption. The amount of oxygen consumed by the kidney or kidneys was calculated by the formula:

$$O_2 \text{ consumption in cubic centimeters per minute} = \frac{(S_A - S_R) \times [Hb]_A \times F}{100}$$

F = renal blood flow in cubic centimeters per minute, calculated as outlined in the preceding section.

$[Hb]_A$ = hemoglobin content of arterial blood in terms of cubic centimeters O_2 capable of combining with the Hb in 100 cc. of blood.

$[O_2]$ = oxygen content of blood in volumes per cent.

$S = \frac{[O_2]}{[Hb]}$ = saturation of blood with O_2 = fraction of the total oxygen-combining capacity of the hemoglobin which is represented by the O_2 present.

Subscripts A and R indicate arterial and renal venous blood respectively

$$O_2 \text{ consumption per gram of kidney} = \frac{\text{Total } O_2 \text{ consumption}}{W_K}$$

W_K = weight in grams of the kidney or kidneys of the animal.

Example: $[Hb]_A$ = 21.00 vol. per cent O_2 capacity, $S_A = 0.95$, $S_R = 0.80$, $F = 200$, W_K = 60 grams (for 2 kidneys).

$$O_2 \text{ consumed by both kidneys} = \frac{0.15 \times 21.0 \times 200}{100} = 6.3 \text{ cc. per minute}$$

$$O_2 \text{ consumed per gram kidney tissue} = \frac{6.3}{60} = 0.105 \text{ cc. per minute.}$$

The formula for calculating oxygen consumption differs from that usually employed by previous authors, who have based the calculated oxygen consumption of a tissue on the difference between the oxygen *contents* of the arterial blood and the venous blood flowing from the tissue, without consideration of differences in hemoglobin concentration. Such calculation assumes that the hemoglobin concentration is unaffected by volume changes occurring while the blood flows through the tissue, or by such difference in time as may occur between the drawing of the arterial and venous blood samples. In fact, however, the blood concentration differences which can occur, at least in the case of the kidney, are such that serious error would frequently result from failure to include the changes in the calculation.

For example, take the period between the first and second catheterizations of the experiment given in table 1. The oxygen taken by the kidney from each cubic centimeter of blood, if calculated merely as the difference between the arterial and venous O_2 contents, would be 0.2083 − 0.1834 = 0.0249 cc. However, the hemoglobin content of the arterial blood is represented by 23.00 volumes per cent O_2 capacity, while that of the renal blood is only 21.84. A considerable part of the decrease in oxygen concentration in the latter is therefore due, not to removal of oxygen by the kidney, but to dilution of the hemogobin. The arterial oxygen saturation, S_A, was 20.83/23.00 = 0.906, and the renal venous blood saturation, S_R, was 18.34/21.84 = 0.840. The actual oxygen removed was therefore 0.906 − 0.840 = 0.066 of the O_2 capable of being bound by the hemoglobin; $0.066 \times \frac{23.00}{100}$ = 0.0152 cc. of O_2 taken from each cubic centimeter of arterial blood which enters the kidney. The deviation of this value from the 0.0249 cc., calculated from the simple difference between arterial and venous O_2 contents, exemplifies the error that may arise from failure to take into account changes in hemoglobin concentration.

For calculating oxygen consumption per gram of kidney, the weight of the kidney has been estimated as described in connection with the calculation of renal blood flow.

Examples of experiments. Space prevents giving the detailed results period by period for each experiment. We have therefore selected 3 pairs of experiments which are representative and given the results graphically in figures 1, 2, and 3. Each pair consists of one experiment in which no

TABLE 1

Protocol of experiment with urea administration

Dog CO (one kidney explanted, one removed)

Italics indicate blood values interpolated for mid-point of each period of urine collection. Brackets enclose arterial urea contents interpolated for time of renal venous blood drawing.

TREATMENT	TIME	BLOOD UREA N		BLOOD O_2 CAPACITY		BLOOD O_2 CONTENT		URINE FOR PERIOD	
		Arterial	Renal venous	Arterial	Renal venous	Arterial	Renal venous	Volume	Urea N
		mgm. per cc.	*mgm. per cc.*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*cc.*	*mgm. per cc.*
Urea intravenous, 1.5 gm. per kgm.	9:28								
	9:41								
	10:40	[0.963]	0.874		20.38		17.85		
	10:43			22.79		21.18			
	10:49	0.948							
Catheterization 1	11:01								
200 cc. 0.3 per cent saline by stomach tube	11:04								
	11:21	[0.887]	0.812		21.83		18.33		
Period 1	*11:24*	*0.882*	*0.806*	*23.00*	*21.84*	*20.83*	*18.34*		
	11:26	0.878				20.77			
	11:35			23.03					
Catheterization 2	11:47½							135	6.59
Period 2	*12:11*	*0.805*	*0.723*	*23.25*	*22.31*	*21.28*	*18.51*		
	12:21	[0.792]	0.706		22.43		18.55		
	12:25	0.782		23.34		21.47			
Catheterization 3	12:35							142	7.52
200 cc. 3 per cent saline by stomach tube	12:36								
	12:48	[0.737]	0.666		22.34		17.70		
Period 3	12:52	0.731		22.26		21.02			
	12:53½	*0.729*	*0.653*	*22.26*	*22.21*	*21.03*	*17.66*		
Catheterization 4	1:12							124	6.38
	1:38	[0.667]	0.581		21.38		17.37		
	1:43	0.662		22.29		21.24			

For renal blood flow, oxygen consumption, and urea clearance values calculated from these data, see tables 4 and 6, dog CO.

urea was given, and another on the same animal in which enough urea was given to raise the blood urea to 5 or 10 times the usual level. For one pair of these experiments the complete analytical data are also given, in tables 1 and 2.

TABLE 2

Protocol of experiment without urea administration

Dog CO (one kidney explanted, one removed)

Italics indicate blood values interpolated for mid-point of each period of urine collection. Brackets enclose arterial urea contents interpolated for time of renal venous blood drawing.

TREATMENT	TIME	BLOOD UREA N		BLOOD O_2 CAPACITY		BLOOD O_2 CONTENT		URINE FOR PERIOD	
		Arterial	Renal venous	Arterial	Renal venous	Arterial	Renal venous	Volume	Urea N
		mgm. per cc.	*mgm. per cc.*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*cc.*	*mgm. per cc.*
	8:58	[0.0849]	0.0784		24.18		20.93		
	9:02	0.0850		24.48		23.16			
Catheterization 1	9:16								
150 cc. 0.3 per cent saline	9:17								
Period 1	*9:35*	*0.0858*	*0.0801*	*23.00*	*22.21*	*21.96*	*18.48*		
	9:36	[0.0859]	0.0802		22.16		18.41		
	9:45	0.0861		22.55		21.60			
Catheterization 2	9:54							41	1.087
	10:03	[0.0874]	0.0806		22.51		18.75		
	10:08	0.0878		23.84		22.56			
Period 2	*10:10*	*0.0879*	*0.0807*	*23.76*	*22.31*	*22.48*	*18.65*		
150 cc. 0.3 per cent saline	10:12								
Catheterization 3	10:26							77	0.668
	10:30	[0.0892]	0.0811		21.79		18.38		
	10:34	0.0894		22.84		21.55			
Period 3	*10:41½*	*0.0901*	*0.0809*	*22.69*	*21.97*	*21.36*	*18.36*		
Catheterization 4	10:57							71	1.112
	11:01	[0.0920]	0.0807		22.28		18.33		
	11:04	0.0922		22.24		20.83			
Period 4	*11:11½*	*0.0915*	*0.0817*	*22.46*	*22.70*	*20.90*	*18.60*		
Catheterization 5	11:26							68	0.692
	11:28	[0.0899]	0.0837		23.38		18.96		
	11:31	0.0896		23.04		21.05			

For renal blood flow, oxygen consumption, and urea clearance values calculated from these data, see table 4, dog CO.

RESULTS. *The proportion of urea extracted from the blood by the kidneys.*[3] Picard in 1856 found that blood from the renal vein of the dog contained

[3] The term "extraction" was introduced by Dunn, Key and Sheehan (1931) for the removal of urea from the blood by the kidneys. We have adopted it in that sense, and shall call the percentage of arterial blood urea, removed during passage of the blood through the kidneys, the percentage extraction.

only half as much urea as the arterial blood. So far as the writers are aware, no other data from the dog are in the literature. Addis and Shevky (1917) in rabbits found that the kidney removed −1 to 23 per cent of the urea from the blood. Dunn, Kay, and Sheehan (1931) found that the

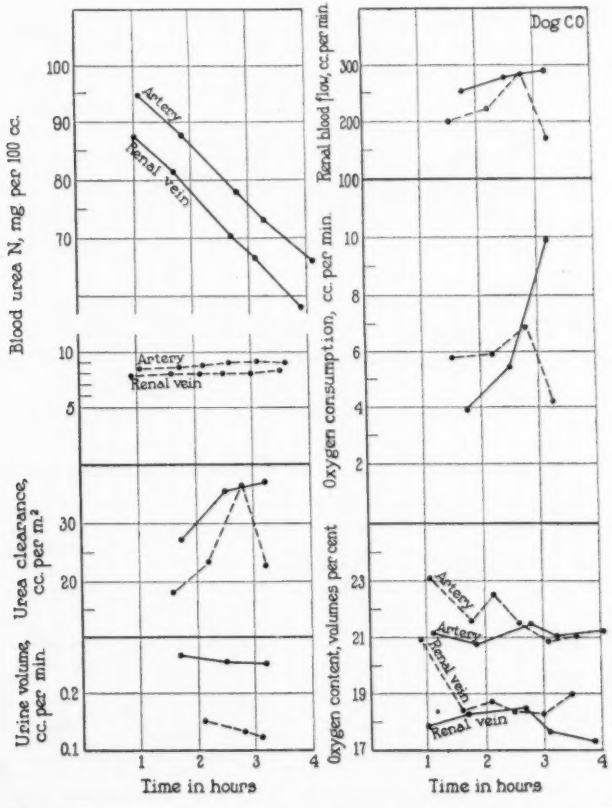

Fig. 1. Dog CO, one kidney explanted, the other kidney removed. The chart represents two separate experiments, one in which 0.70 gram of urea nitrogen per kilo were given intravenously before the experimental period and one in which no urea was given.

——— Urea given.

- - - - No urea given.

kidney of the rabbit usually extracted between 6 and 13 per cent, but that in some cases the urea removal was so little that, as in certain of Addis and Shevky's experiments, it was within the limits of analytical error. Dunn, Kay and Sheehan were of the impression that in these cases the kidneys

were not acting normally. Both these authors and Addis and Shevky obtained the renal blood by operation on anesthetized animals. Kay and Sheehan (1933) have recently published additional interesting data, which will be discussed later.

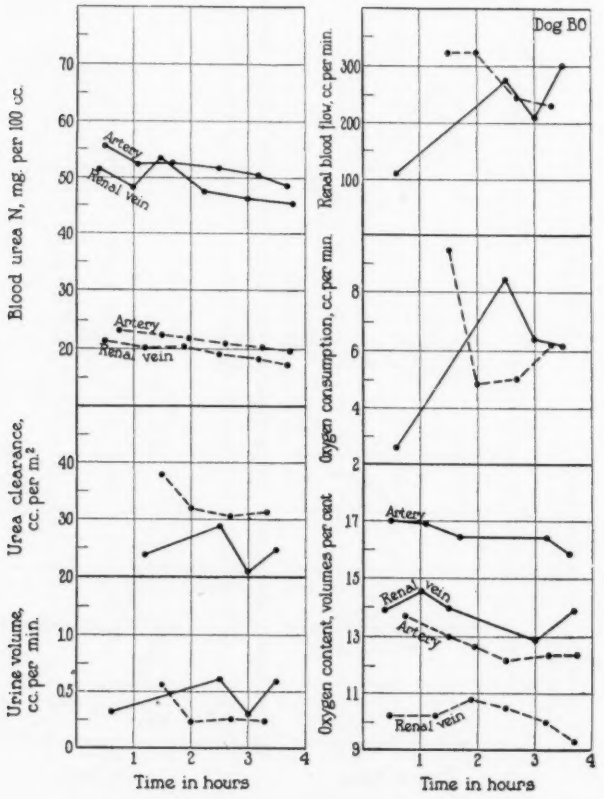

Fig. 2. Dog BO, one kidney explanted, the other kidney removed. The chart represents two separate experiments, one in which 0.23 gram of urea nitrogen per kilo was given intravenously before the experimental period and one in which no urea was given.

Note in upper left curves cessation of urea removal during second hour.

———— Urea given.

- - - - No urea given.

Our results show that Picard's figure of 50 per cent urea extraction is far too high, and that the extraction in the dog is practically the same found

by Addis and Shevky, Dunn, Kay and Sheehan, and Kay and Sheehan[4] in the rabbit. The experiments on dog CO shown in figure 1 and table 4 represent, we believe, data obtained under conditions nearly ideal. The

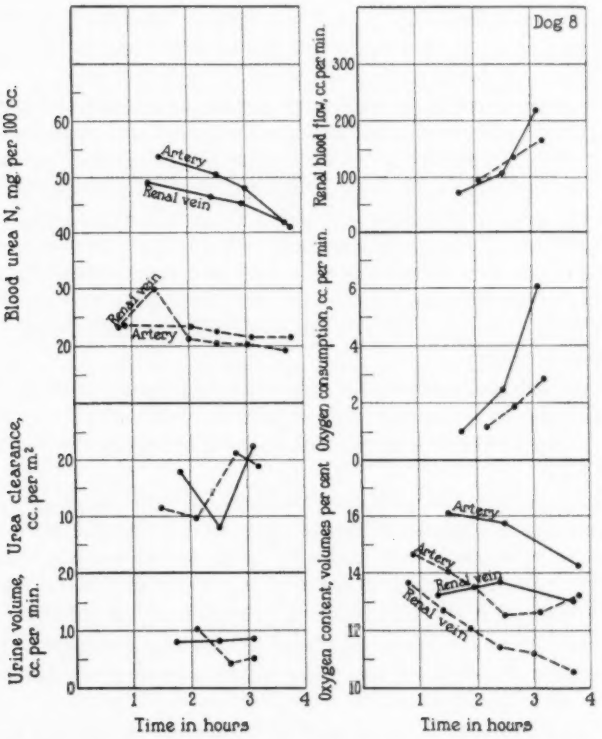

Fig. 3. Dog 8, one kidney explanted, the other kidney removed. The chart represents two separate experiments, one in which 0.23 gram of urea nitrogen was given by stomach tube before the experimental period and one in which no urea was given. Return of urea from kidney to renal vein occurred in second hour of experiment without urea administration.

——— Urea given.

- - - - No urea given.

animal was trained to such experiments, and was not disturbed by being catheterized and having blood drawn. The arterial and venous urea curves

[4] During the first minute after intravenous injection into rabbits of urea sufficient to raise the blood concentration momentarily to the neighborhood of 2 per cent, Kay and Sheehan found that 20 to 40 per cent of the urea carried by the blood to the kidneys remained in them. The urea thus held by the kidneys, however, must represent

in figure 1 indicate freedom from irregularity. In the experiment where blood urea was increased about 10-fold by urea administration, urea extraction varied from 8.6 to 10.4 per cent of the arterial content. When no urea was given, and the arterial urea level was about one-tenth as high, the urea extraction varied from 6.7 to 10.6 per cent of the arterial content. *The proportion of urea extracted was practically the same, whether the arterial urea content was at the usual fasting level or at 10 times that level.*

With this fact the first question proposed in our introduction is answered. *It is the ability of the normal kidney to remove from each cubic centimeter of blood a constant proportion of the urea present, whether the concentration is high or low, that enables the organ to clear of urea the same volume of blood per minute, despite many-fold variations in urea content.* This ability is maintained over all ranges of blood urea concentration that we have studied (8 to 137 mgm. urea N per 100 cc.). Therefore no other adjustment of the kidney's function, such as change in blood flow, is necessary to maintain the urea clearance within its usual limits in the face of great variations in blood urea content.

The urea extraction figure for one human subject, a case of malignant nephrosclerosis, has recently been reported by Weiss, Parker and Robb (1933). They found an extraction of 10 per cent, nearly the average of our normal dogs.

The range of variation in the percentage of arterial blood urea extracted during passage through the kidney, in dogs with either one or two kidneys, is indicated by tables 3 and 4, and by figure 4. It is evident that in the majority of the observations between 6 and 12 per cent of the arterial urea was extracted from the blood during perfusion of the kidney. The few urea extractions below 5 per cent probably represent instances in which the urea-excreting function of the kidney was momentarily diminished or

not only that removed by the renal excretory mechanism, but also that which passes by physical diffusion from the highly charged blood into the renal tissues, as it does into the muscles and other non-renal tissues. The amount of urea taken from the blood per gram tissue during the minute after injection is indicated by Kay and Sheehan's data to be several times as great in the kidneys as in the general tissue: This fact, however, could be expected from the similarly more rapid flow of blood per gram of tissue through the kidneys. During the period 10 to 30 minutes after the injection, Kay and Sheehan found urea returning from the kidneys to the blood, the concentration being higher in the renal vein than in the artery. Some 50 minutes after the injection diffusion equilibrium among blood, kidneys, and other tissues appeared to be approximated, and the percentage urea extraction regained its usual value of 5 to 8. The phenomena seem to occur most probably in accord with the explanation that, of the urea taken up by the kidneys during the first minute after such injections, half or more entered the kidneys by simple diffusion into the renal tissues, and could not be considered as taken out of the blood by the excretory processes of the organ.

suspended by the disturbances attending catheterization or blood drawing. In four instances such effects were made obvious by the fact that the renal venous blood actually contained more urea than the arterial blood, indicating that for the moment reabsorption of urea from the kidney into the blood replaced excretion.

Kay and Sheehan (1933), in an important paper which appeared after the present work had been prepared for press, report data indicating that there is a tendency for the percentage of blood urea extracted by the kidneys to fall somewhat when the blood urea concentration rises to levels at which we failed to note such a tendency. Experimenting with rabbits in which the kidneys were bared under urethane narcosis, these authors determined the renal blood flow by direct measurement of the blood which escaped from the severed renal vein. They estimated the percentage of urea extracted from the blood passing the kidneys by two methods, one of which was comparison of the urea content of arterial and renal blood, the procedure used in this paper. Their values for percentage urea extraction were mostly, like ours, between 6 and 10, but an averaging curve (their fig. 6) relating extraction to blood urea concentration, showed a distinct tendency for the *percentage* of urea extracted to fall as the blood urea rose. Their curve indicates a mean extraction of about 9 per cent when the blood urea was normal and about 5 per cent when injection raised the blood urea N about 10-fold to 120 mgm. per 100 cc. Their renal blood flows showed no change, so that the clearances must have paralleled the percentage extraction values. Our data from 51 determinations in table 4 show the following distribution: When blood urea nitrogen concentrations are separated into 3 groups, with 8 to 50, 50 to 100, and 100 to 137 mgm. of urea N per 100 cc. of blood, the corresponding average urea extractions are 9.4, 9.5, and 8.4 per cent. The number of observations in the 3 groups were, respectively, 34, 14, and 5. The individual extractions for the group with over 100 mgm. of blood urea N per 100 cc. were 6.7, 6.9, 9.3, 9.5, and 9.9 per cent. The fact that the average was 8.4 compared with 9.4 for the low blood urea group can not be taken as significant. Our results may be taken to indicate definitely that raising the blood urea N concentration of dogs up to 100 mgm. per cent does not alter the per cent of urea extracted by the kidneys. Above this level our data are not numerous enough to be conclusive, but indicate the probability of no alteration up to 137 mgm. per cent, where an extraction of 9.9 per cent was noted.

The difference between Kay and Sheehan's results on this point and ours may be attributable to one of the following causes: 1. They used rabbits, and we, dogs. 2. Their animals were anesthetized and operated while ours were in normal condition. The latter cause seems the more likely, because Drury (1923) increased the blood urea of normal rabbits to much higher levels (over 300 mgm. urea N per 100 cc.) than in Kay and Sheehan's experiments, without decreasing the urea clearance. It is probable that Drury's constant clearance indicated also a constant percentage urea extraction: if the percentage extraction had decreased, the renal blood flow would have had to increase by the same proportion to keep the clearance constant. On the contrary, Kay and Sheehan's data (their table 12) show no effect of urea injection on renal blood flow in rabbits, nor do our data in dogs. It appears probable that, despite the apparently good renal function maintained in Kay and Sheehan's animal preparations, they were enough affected by the operation and anesthetic to fall somewhat below intact animals in the ability to maintain a constant percentage urea extraction in the face of elevated blood urea concentration.

TABLE 3

Percentage of arterial blood urea removed by explanted kidney in 2-kidney dogs

DOG NO.	N ADMINISTERED PER KGM.	FIRST PERIOD		SECOND PERIOD	
		Urea N in arterial blood per 100 cc.	Per cent of arterial urea removed by kidney	Urea N in arterial blood per 100 cc.	Per cent of arterial urea removed by kidney
	grams	*mgm.*	*per cent*	*mgm.*	*per cent*
D1	None	15.30	10.4	14.45	10.7
D4	None	19.00	10.3	18.35	9.8
D9	None	16.50	2.6	15.67	7.9
D9	None	11.11	5.9	11.16	3.8
E4	None	8.90	5.4	9.10	0.7
E4	None	8.40	19.3	9.39	−0.3
E5	None	7.10	8.7	6.89	7.1
E5	None	18.50	11.9	15.04	13.2
I0	Meat 0.83	15.17	12.0		
A8	Meat 0.86	13.56	14.0		
B0	Urea 0.23	28.35	8.6		

TABLE 4

Percentage of arterial blood urea removed by explanted kidney in 1-kidney dogs

Arterial values are interpolated to time of renal vein puncture

DOG NO.	INTRAVENOUS UREA N PER KGM.	FIRST PERIOD		SECOND PERIOD		THIRD PERIOD		FOURTH PERIOD		FIFTH PERIOD		SIXTH PERIOD	
		Urea N in arterial blood per 100 cc.	Per cent of arterial urea removed by kidney	Urea N in arterial blood per 100 cc.	Per cent of arterial urea removed by kidney	Urea N in arterial blood per 100 cc.	Per cent of arterial urea removed by kidney	Urea N in arterial blood per 100 cc.	Per cent of arterial urea removed by kidney	Urea N in arterial blood per 100 cc.	Per cent of arterial urea removed by kidney	Urea N in arterial blood per 100 cc.	Per cent of arterial urea removed by kidney
	gram	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*
B9	0.93	137.0	9.9	124.1	6.9	115.1	6.7	107.8	9.3	100.5	9.5	95.7	9.5
A8	0.14	43.30	11.8	39.15	10.2	36.30	6.6						
8	0.23	53.90	9.1	50.80	8.7	48.20	6.4	42.20	1.0				
8	None	23.50	0.6	23.45	−27.5	23.25	9.2	22.64	9.4	21.53	6.2	21.40	9.4
B0	0.23	56.30	9.1	52.60	8.6	52.50	−1.3	51.93	8.1	50.65	8.4	48.90	6.3
B0	None	23.40	8.6	22.57	11.0	21.97	7.2	21.15	9.5	20.30	9.5	19.90	10.6
C0	0.70	96.30	9.2	88.73	8.5	78.60	10.3	73.70	9.8	66.70	13.0		7.0
C0	None	8.47	7.4	8.58	6.5	8.73	7.7	8.90	8.9	9.18	12.1	9.00	
I0	0.23	39.40	9.6	39.30	8.9	39.50	9.3	40.25	9.6	39.50	8.9		
B7	None	31.25	14.9	29.40	9.2								
B7	None	22.47	9.5	21.50	9.1								
C1	None	20.20	6.9	20.02	2.9								
C1	None	15.32	7.0	15.13	0								

Reabsorption of urea from the kidney into the blood. The upper pair of blood urea curves of figure 2 shows one period when the usual difference between the urea contents of venous and arterial blood suddenly disappears. In figure 3 a similar phenomenon occurs in one period, but to a more exaggerated degree, the venous blood having actually 27 per cent more urea than the arterial. This phenomenon was encountered several times in our experiments. It usually affected only one observation in a

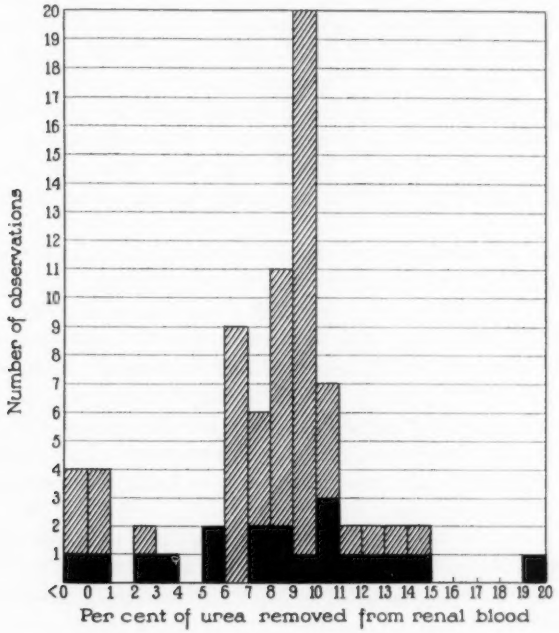

Fig. 4. Distribution curve of percentages of urea removed from blood passing through kidney. The blackened areas indicate observations on two-kidney dogs, the unexplanted kidney being *in situ*. The shaded areas indicate observations on one-kidney dogs.

series, periods immediately before and after such an observation showing the usual 6 to 12 per cent urea removal. The reabsorption of urea was not attributable to stoppage of blood flow; renal blood, the increased urea content of which demonstrated the occurrence of reabsorption at the moment, was drawn from the renal vein with as much facility as usual, and the proportion of oxygen removed from it by the kidney was quite normal.

It is our belief that the phenomenon was due to some reflex initiated at times by slight trauma connected with puncture of the renal vein. We

attempted to block such reflexes by cocainizing the region where the puncture was made, but without success. Neither could we at will produce the effect by intentional trauma. The mechanism of the phenomenon remains a riddle, but the fact of its occurrence is of interest in showing that the excretory mechanism of the kidney can be so easily influenced by stimuli from without the organ, and furthermore that reabsorption of urea in the kidney can occur.

There is a possibility that the apparent reabsorption of urea was an artifact, due to admixture of slight amounts of urine with the renal venous blood. Such admixture might conceivably occur if the needle punctured the ureter before it reached the renal vein. However, the technique used would make it difficult for such an occurrence to escape observation. The blood was drawn through a glass capillary tube, which was watched in order to note, by the start of blood flow, when the vein was entered. A few cubic millimeters of urine preceding the blood would have been visible.

THE RENAL BLOOD FLOW AND ITS RELATIONS TO UREA CLEARANCE, PERCENTAGE OF BLOOD UREA EXTRACTED AND URINE FLOW. *The rate of renal blood flow.* The rate of renal blood flow was determined in 11 experiments on two-kidney dogs and 13 experiments on one-kidney dogs. In each set of experiments 8 different animals were used. Each experiment consisted of 3 or 4 consecutive periods of 40 to 60 minutes each. In tables 5 and 6 results period by period are given for the experiments, which indicate the extent of variation that was encountered. Table 7 gives average results from one- and two-kidney dogs. Table 8 summarizes blood flow data of other authors.

Effect of removing one kidney on the blood flow and urea clearance through the remaining kidney. Table 7 indicates that after removal of one kidney the blood flow through the other is markedly increased. Herrick, Essex, and Baldes (1932) found, on the contrary, that after removal of one kidney the blood flow through the other continued unaltered. However, their observations extended for only a period of about three hours after the unilateral nephrectomy, while our post-operative observations did not begin until a week after removal of the unexplanted kidney. It appears, therefore, from comparison of their results and ours, that increased perfusion of the remaining kidney does not begin for several hours after unilateral nephrectomy but that within a period of a week or more the blood flow through the remaining kidney does undergo so marked an increase that it does not fall far short of the flow through both kidneys before the operation.

With the increased blood flow through the remaining kidney, the volume of blood cleared of urea by it also increases. After removal of one kidney the average perfusion per gram of the remaining kidney increased to 168

TABLE 5

Renal blood flow, oxygen consumption and blood urea clearance of dogs with 2 kidneys, one explanted and one left in situ§

DOG NO.	BODY WEIGHT	SURFACE AREA	ESTIMATED WEIGHT OF BOTH KIDNEYS	N ADMINISTERED PER KGM.	PERIOD NO.	RENAL BLOOD FLOW		RENAL O_2 CONSUMPTION		BLOOD CLEARED OF UREA PER MINUTE		UREA REMOVED BY KIDNEY
						Per minute	Per gm. kidney per minute	Per minute	Per gm. kidney per minute	Per m^2 body surface	Per gm. kidney	
	kgm.	*m^2*	*grams*	*grams*		*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*
D1	15.7	0.690	66.4*	None	1	200	3.31	6.52	0.098	32.9	0.34	11.4
					2	246	3.70	5.32	0.080	37.5	0.39	10.5
D4	17.1	0.693	66.7*	None	1	253	3.79	6.18	0.093	36.8	0.38	10.1
					2	241	3.61	3.97	0.060	33.8	0.35	9.7
D9	12.0	0.554	53.3*	None	2	213	4.00	2.51	0.047	28.2	0.29	7.4
D9	12.0	0.554	53.3*	None	1	392	7.35	5.86	0.110	42.0	0.44	5.9
E4	10.6	0.536	60.0*	None	1	279	4.65	8.62	0.144	29.0	0.26	5.5
E4	10.6	0.536	60.0*	None	1	116	1.93	1.99	0.033	42.4	0.38	19.7
E5	16.4	0.668	120.0†	None	1	547	4.56	13.28	0.111	70.2	0.39	8.6
					2	224	1.87	6.08	0.051	23.2	0.13	6.9
E5	16.4	0.668	120.0†	None	1	338	2.82	9.38	0.078	58.0	0.32	11.4
					2	306	2.55	9.80	0.082	58.4	0.32	12.7
10	11.0	0.520	48.0†	Meat 0.83 before expt.	1	235	4.90	3.11	0.065	54.2	0.59	12.0
					2	243	5.04	—¶	—	56.0	0.61	12.0
A8	10.5	0.580	52.5†	Meat 0.86 before expt.	1	286	5.44	4.53	0.086	68.8	0.76	14.0
					2	181	3.45	—¶	—	43.6	0.48	14.0
B0	17.2	0.800	89.0†	Urea 0.23 by stomach tube before expt.	1	457	5.14	6.16	0.069	48.8	0.44	8.6
					2	356	4.00	—¶	—	38.0	0.34	8.6

For average of results in table 5 see table 7.

* Estimated as 96.2 × square meters body area. Stewart (1921) has shown that kidney weight in dogs varies directly as the surface area. Taylor, Drury and Addis (1923) have shown that a similar relationship holds in rabbits, and MacKay (1932) has found it in man. Body surface was calculated by length-weight formula of Cowgill and Drabkin (1927).

† Estimated as twice the weight of one kidney subsequently removed.

‡ Urea removed by kidney was so little that reflex interference appeared probable. Blood flow and O_2 consumption therefore were not calculated.

¶ No analyses.

§ Blood values are from data interpolated for the mid-point of each urine collection period.

TABLE 6

Renal blood flow, oxygen consumption and blood urea clearance of dogs with only 1 kidney each (explanted)§

DOG NO.	BODY WEIGHT	SURFACE AREA	WEIGHT OF REMOVED KIDNEY	UREA N ADMINISTRATION	PERIOD NO.	RENAL BLOOD FLOW		RENAL O_2 CONSUMPTION		BLOOD CLEARED OF UREA PER MINUTE		UREA REMOVED BY KIDNEY
						Per minute	Per gm. kidney per minute	Per minute	Per gm. kidney per minute	Per m^2 body surface	Per gm. kidney	
	kgm.	*m^2*	*grams*	*grams*		*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*
B9	8.40	0.449	22.40	0.93 intravenously before experiment	1	177	7.90	3.78	0.169	26.0	0.52	6.6
					2	240	10.70	5.13	0.229	28.4	0.57	5.3
					3	155	6.92	3.24	0.145	26.8	0.54	7.8
					4	105	4.69	2.35	0.105	30.8	0.62	13.2
A8	12.10	0.606	26.27	0.14 intravenously before expt.	1	202	7.69	4.92	0.187	37.4	0.86	11.2
					2	247	9.40	—*	—	37.4	0.86	9.2
					3	260	9.90	5.32	0.203	30.4	0.70	7.1
8	12.25	0.558	28.40	0.23 by stomach tube before expt.	1	73	2.57	1.12	0.039	18.0	0.36	13.8
					2	108	3.80	2.47	0.087	8.3	0.16	4.3
					3	220	7.75	6.04	0.212	22.8	0.46	5.8
8	13.20	0.574	28.40	None	1	—†	—	—	—	—	—	—
					2	91	3.20	1.19	0.042	15.2	0.31	9.6
					3	139	4.90	1.89	0.067	21.3	0.43	8.8
					4	166	5.84	2.81	0.099	18.9	0.38	6.5
B0	16.50	0.791	44.50	0.23 intravenously before expt.	1	110	2.47	2.61	0.059	11.7	0.21	8.4
					2	—‡	—	—	—	—	—	—
					3	—‡	—	—	—	—	—	—
					4	278	6.24	8.42	0.189	29.0	0.52	8.3
					5	210	4.72	6.39	0.143	21.2	0.38	8.0
					6	303	6.81	6.18	0.139	24.9	0.44	6.5
B0	16.50	0.791	44.50	None	1	322	7.24	9.52	0.214	38.4	0.68	9.4
					2	323	7.26	3.88	0.087	32.1	0.57	7.9
					3	247	5.55	5.02	0.113	30.7	0.55	9.8
					4	233	5.23	6.21	0.140	31.7	0.56	10.8
C0	17.75	0.780	32.20	0.70 intravenously before expt.	1	252	7.82	3.82	0.119	27.8	0.66	8.6
					2	274	8.51	5.41	0.168	35.8	0.87	10.2
					3	282	8.75	9.40	0.292	37.6	0.91	10.4

For average of results of table 6 see table 7.

* No analyses obtained.

† Urea content of renal vein heigher than arterial.

‡ Urea removal by kidney was so slight that reflex interference appeared probable. Blood flow and O_2 consumption therefore were not calculated.

§ Blood values are calculated from data interpolated for the moment at the midpoint of each urine collection period.

TABLE 6—*Concluded*

DOG NO.	BODY WEIGHT	SURFACE AREA	WEIGHT OF REMOVED KIDNEY	UREA N ADMINISTRATION	PERIOD NO.	RENAL BLOOD FLOW		RENAL O_2 CONSUMPTION		BLOOD CLEARED OF UREA PER MINUTE		UREA REMOVED BY KIDNEY
						Per minute	Per gm. kidney per minute	Per minute	Per gm. kidney per minute	Per m^2 body surface	Per gm. kidney	
	kgm.	m^2	*grams*	*grams*		*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*
C0	17.50	0.775	32.20	None	1	206	6.40	5.83	0.181	17.7	0.43	6.7
					2	223	6.92	5.88	0.183	23.6	0.57	8.2
					3	277	8.60	6.66	0.207	36.5	0.88	10.2
					4	166	5.15	4.14	0.129	22.8	0.55	10.6
10	10.80	0.520	24.00	0.23 by stomach tube before expt.	1	184	7.67	—	—	33.0	0.72	9.3
					2	213	8.87	—	—	34.4	0.75	8.4
					3	216	9.00	3.23	0.135	36.5	0.79	8.8
					4	207	8.62	—	—	38.2	0.83	9.6
B7	10.20	0.466	21.11	None	1	84	3.98	2.23	0.106	24.0	0.53	13.2
					2	144	7.82	3.59	0.170	25.5	0.56	8.2
B7	10.20	0.466	21.11	None	1	68	3.22	1.60	0.076	13.3	0.29	9.1
					2	188	8.90	4.80	0.227	29.7	0.66	7.4
C1	17.15	0.729	31.50	None	1	181	5.74	3.18	0.101	17.2	0.40	6.9
C1	17.90	0.735	31.50	None	1	266	8.45	5.76	0.183	25.6	0.60	7.1

per cent, and the average urea clearance to 143 per cent, of the preoperative values. (See discussion of these phenomena in the preceding paper.)

After the first observations, about a week following operation, we observed little tendency to further increase in renal blood flow or clearance.

Relation of renal blood flow to urea clearance. Figure 5 shows that the urea clearance parallels the renal blood flow. It appears that, in the normal dog, change in renal blood flow is the main physiological factor which determines changes in urea clearance. A preliminary report by Medes, Herrick, and Baldes (1932) states that they have similarly found in dogs that the creatinine clearance (which they term "glomerular filtrate") parallels the renal blood flow, which was measured by stromuhr.

If the percentage of urea extracted from the blood by the kidney were entirely constant, the clearance would by mathematical necessity be exactly proportional to the renal blood flow, and all the points in figure 5 would lie on one line. The degree of scattering observed is attributable to the varia-

tions in percentage urea extraction, which have been shown in figure 4. Comparison of figure 5 with figure 6 shows, however, that variation in percentage urea extraction is ordinarily a minor factor, compared with renal blood flow, in determining the urea clearance in the normal animal.

Relation of renal blood flow to percentage urea extraction. One might anticipate the possibility that when the blood flows more rapidly through the kidney, the process by which urea is removed might be less complete. However, figure 7 shows no indication of any such effect, within the limits of spontaneously occurring variations in blood flow. It appears that, whatever processes are concerned with the removal of urea from the blood, they

TABLE 7

Comparison of mean values for renal blood flow, oxygen consumption and urea clearance in 1- and 2-kidney dogs

	RENAL BLOOD FLOW		RENAL O_2 CONSUMPTION		BLOOD CLEARED OF UREA PER MINUTE		UREA REMOVED BY KIDNEY
	Per minute	Per gm. kidney per minute	Per minute	Per gm. kidney per minute	Per m^2 body surface	Per gm. kidney	
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*
Two-kidney dogs average total	284	4.01	6.21	0.080	44.6	0.40	10.5
One-kidney dogs:							
Average total	201	6.72	4.54	0.145	26.9	0.57	8.7
Per cent of average of two-kidney dogs	71	168	73	181	61	143	8.3
Average with urea administration	206	7.18	4.70	0.154	28.4	0.61	8.6
Average without urea administration	195	6.14	4.38	0.137	25.0	0.52	8.8

approach equilibrium with such rapidity, that even the most rapid normal flow does not get the blood through the organ before the extraction has become as complete as other forces permit.

Relation of renal blood flow to diuresis. The early workers, Landergren and Tigerstedt (1893) and Gottlieb and Magnus (1901), reported an increase in renal blood flow during the diuresis produced by urea administration. Other workers, Schwarz (1899), Barcroft and Brodie (1904–5, and 1905–6), Lamy and Mayer (1906), Brodie (1913–14), Tamura and Miwa (1919–20), Cushny and Lambie (1921), Dreyer and Verney (1922–23) and Glaser, Laszlo, and Schürmeyer (1932 and 1933) found no correlation between renal blood flow and the diuresis produced by administration of urea.

TABLE 8

Summary of previous data on rates of blood flow through one kidney of the dog

AUTHOR	DATE	PREPARATION OF ANIMAL	METHOD OF MEASURING BLOOD FLOW	RENAL BLOOD FLOW OBSERVED					
				Total per minute			Per gm. kidney per minute		
				Max.	Min.	Mean	Max.	Min.	Mean
				cc.	cc.	cc.	cc.	cc.	cc.
Bainbridge and Evans	1914	Heart-lung-kidney preparation	Direct measurement						
Dreyer and Verney	1922-23	Heart-lung-kidney preparation	Direct measurement	250	105				
Starling and Verney	1924-25	Heart-lung-kidney preparation	Direct measurement	380	72	154	5.3	3.0	4.1
Fee and Hemingway	1928	Heart-lung-kidney preparation	Direct measurement	135	68				
Gremels	1929	Heart-lung-kidney preparation	Direct measurement	288	136	197			
Verney	1929	Heart-lung-kidney preparation	Direct measurement	230	128	175			5.2
Canny, Verney and Winton	1929-30	Heart-lung-kidney preparation	Direct measurement	117	67	98			
Verney and Winton	1930	Heart-lung-kidney preparation	Direct measurement	214	82	126			
Winton	1931-a	Heart-lung-kidney preparation	Direct measurement	141	75	111			
Winton	1931-b	Heart-lung-kidney preparation	Direct measurement	143	65	104			
Hemingway	1933	Heart-lung-kidney preparation	Direct measurement	134	71	107	6.1	2.2	4.4
Landergren and Tigerstedt	1893	Morphine and atropine and operation	Stromuhr	44.8	6.7	24.4	0.88	0.25	0.49
Schwarz	1899	Anesthesia and operation	Direct measurement	86	23	49			
Barcroft and Brodie	1904-5	Chloroform anesthesia and evisceration	Direct measurement	200	4.0	114.4			
Barcroft and Brodie	1905-6	Chloroform anesthesia and evisceration	Direct measurement	150	8.4	106			
Lamy and Mayer	1906	Chloral anesthesia and operation	Direct measurement	400	92.3	187.1			
Burton-Opitz and Lucas	1908-a	Chloroform and ether anesthesia and operation	Stromuhr	171.6	28.2	98.4	1.86	0.70	1.51
Burton-Opitz and Lucas	1908-b	Chloroform and ether anesthesia and opation	Stromuhr	188	88	145			
Richards and Plant	1922	Urethane and ether anesthesia and operation	Direct measurement	93.7	88.2	90.0			
Janssen and Rein; and Rein	1927-28	Post-operative	Thermo-electric				7.0	1.3	2.5
Hayman and Schmidt	1928	Sodium pheno-barbital anesthesia and operation	Direct measurement				5.3	0.42	

TABLE 8—*Concluded*

AUTHOR	DATE	PREPARATION OF ANIMAL	METHOD OF MEASURING BLOOD FLOW	RENAL BLOOD FLOW OBSERVED					
				Total per minute			Per gm. kidney per minute		
				Max.	Min.	Mean	Max.	Min.	Mean
				cc.	cc.	cc.	cc.	cc.	cc.
Herrick, Essex and Baldes	1932	Ether anesthesia or sodium iso-amytal-ethyl barbiturate	Thermo-stromuhr	177	105	144			
Glaser, Laszlo and Schürmeyer	1932	Numal, chloralose hedonal or amytal anesthesia	Thermo-stromuhr				7.20	0.41	2.66
Our results with 1-kidney dogs	1933	1 kidney removed 1 kidney explanted animal normal	Urea excretion	323	68	201	10.7	2.3	6.7
Our results with 2-kidney dogs. Flow estimated through 1 kidney	1933	1 kidney explanted other *in situ*	Urea excretion	274	58	142	7.4	1.9	4.9

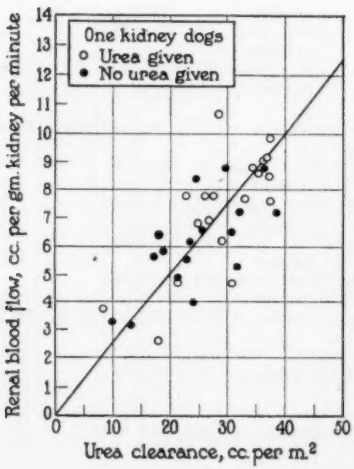

Fig. 5

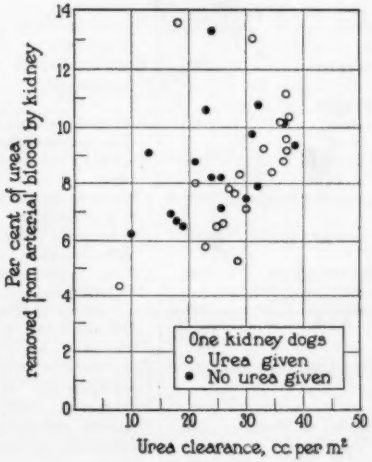

Fig. 6

Fig. 5. Relation of renal blood flow to blood urea clearance
Fig. 6. Relation of percentage blood urea removal to urea clearance

The relation between renal blood flow and rate of urine flow in our experiments is shown in figure 8. Although the urine excretion rate was

greatly increased in most experiments in which urea was given, the renal blood flow was not increased. There is no apparent relation between diuresis and physiological variations in renal blood flow.

RENAL OXYGEN CONSUMPTION AND ITS RELATIONSHIPS TO RENAL BLOOD FLOW, URINE FLOW, AND THE WORK OF UREA EXCRETION. *The rate of oxygen consumption by the kidney.* The rate of renal oxygen consumption in two-kidney and one-kidney dogs is given in tables 5 and 6. Data of previous authors for O_2 consumption by one kidney, in preparations in which the other kidney was presumably present, are summarized in table 9.

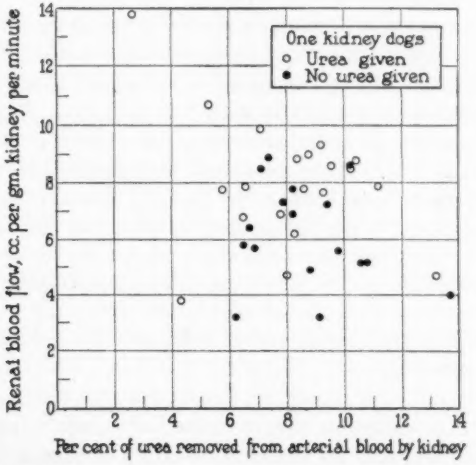

Fig. 7. Relation of renal blood flow to percentage urea removal

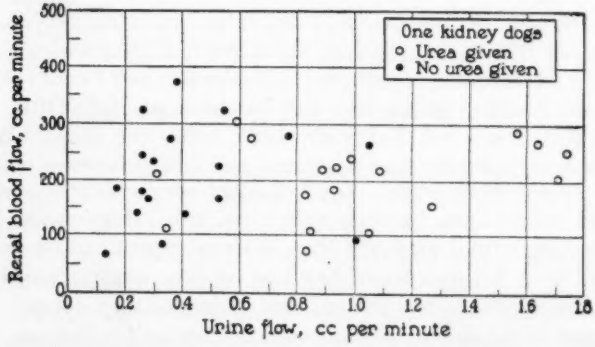

Fig. 8. Relation of renal blood flow to urine flow

Our data agree with those of previous authors in indicating that the oxy-

gen consumption varies within wide limits. In the two-kidney dogs our extreme variations were from 2.0 to 13.3 cc. of O_2 per minute, and in the one-kidney animals from 1.1 to 9.5 cc. per minute. The percentage variations per gram of kidney were not less. In the same animal in the course of an experiment the consumption was seen to change by as much as 6 cc. (e.g., dog CO, fig. 1, and tables 1 and 6).

Effect of removing one kidney on the metabolic rate of the other. Comparison of the mean results for oxygen consumption in table 7 shows that the animals with only one kidney consumed an average of nearly twice as much oxygen per gram as the animals with two kidneys. Apparently after removal of one kidney the metabolic rate of the other is so accelerated that it consumes nearly as much oxygen as had both kidneys previously. How soon after removal of one kidney the oxygen consumption of the other becomes accelerated we do not know, because our first post-operative observations were made about a week after removal of the one kidney. The metabolic acceleration of the surviving kidney apparently occurs within this period, for no marked change was noted thereafter.

The increase in oxygen consumption of the remaining kidney appears to accompany a speeding up of all other activities. The blood flow is increased approximately as much as the oxygen consumption. The urea clearance per gram kidney or square meter body surface, as shown in the preceding paper, and also by the data in tables 5, 6, and 7, is increased likewise, although only about half enough to compensate for the loss of the removed kidney.

The relationship of spontaneous variations in renal blood flow and oxygen consumption. The literature regarding the relationship between blood flow and oxygen consumption is confusing. Bainbridge and Evans (1914) with the heart-lung-kidney preparation and Tamura and Miwa (1919–20) and Tamura, Watanabe, and Kaburaki (1926) using the direct blood flow method on rabbits reported no significant parallelism between renal blood flow and oxygen consumption. Gremels (1929) concluded that oxygen consumption was not a linear function of the blood flow. Hayman and Schmidt (1928), working with dogs, found that no single experiment showed a uniform parallelism between blood flow and oxygen consumption, but that when data on a series of experiments were assembled they showed a considerable degree of parallelism between variations in the two factors. Glaser, Laszlo, and Schürmeyer (1932 and 1933) reported that oxygen consumption increased with renal blood flow as a linear function.

The results of Glaser, Laszlo, and Schürmeyer, for which the blood flow values were obtained by the thermal stromuhr method, are confirmed by ours, in which an altogether different principle was used for the blood flow values. The relationship shown by our data is evident in figure 9.

It appears reasonable to assume that both blood flow and oxygen con-

sumption of the kidney increase in response to the metabolic demands of the organ, rather than that increased blood flow accelerates oxygen consumption, or vice versa. Inspection of results in tables 5 and 6 indicates, in agreement with Hayman and Schmidt (1928), that in the data of a given experiment there may be lack of parallelism between the two factors, the indication of direct proportionality between the two becoming evident only when a considerable number of results are considered. Such a behavior appears to be most probably explained by the assumption that both blood flow and oxygen consumption respond to the same stimulus. In such a case one might expect that a general parallelism in the responses of both

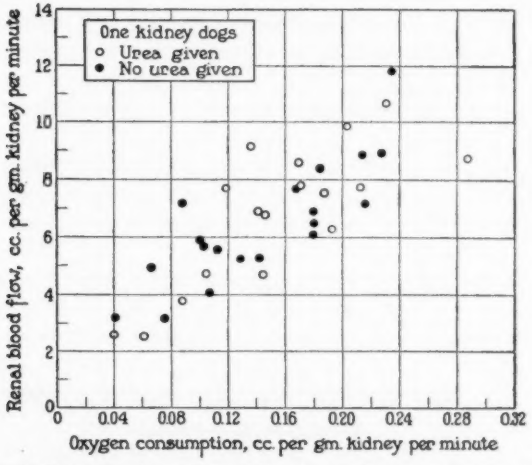

Fig. 9. Relation of renal blood flow to renal oxygen consumption

would occur, but that blood flow and oxygen consumption would not always respond with like alacrity.

Non-relation of renal oxygen consumption to urine flow. The relation between oxygen consumption by the kidney and rate of urine flow in our experiments is shown in figure 10. There is no correlation.

Barcroft and Brodie (1904–5 and 1905–6) and Barcroft and Straub (1910) reported an increase in oxygen consumption as a result of diuresis. Fee and Hemingway (1928) reported a rise in oxygen consumption with increased urine volume. Hayman and Schmidt (1928) found no change in oxygen consumption as a result of urea administration and no correlation between oxygen consumption and concentration or composition of urine. Our results agree with those of Hayman and Schmidt.

Non-relation between renal oxygen consumption and the work of urea excretion. We have sought to find whether a great increase in the excretory

work of the kidney, such as is caused by 10-fold increase in the blood urea content and hence in the rate of urea excretion, causes a significant increase in the oxygen consumption of the organ. Barcroft and Brodie (1905–6) and Glaser, Laszlo, and Schürmeyer (1932) have calculated that the mechanical work which the kidneys do in concentrating and excreting the solid constituents of the urine is less than 1 per cent of the total energy produced by the organ, as measured by its oxygen consumption. However, if the kidney were an uneconomical engine, it might be necessary to consume, for accomplishment of a given unit of work, many-fold the amount of energy calculated for a perfect engine. In such a case the energy consumed would still approach direct proportionality to the thermodynamic work of excretion, if the energy required directly and indirectly for that

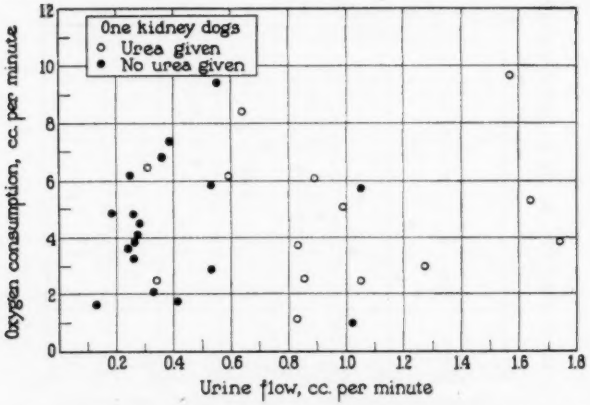

Fig. 10. Relation of renal oxygen consumption to urine flow

work were large compared with that used for the other cellular activities of the organ.

Urea is, of course, not the only urinary constituent contributing to the work of excretion. It is, however, one of the most important, and when it is increased 10-fold the effect on the total excretory work must closely parallel the increase in urea output.

In our results no difference in renal oxygen consumption could be noted between experiments in which urea was administered and those in which it was not. Thus, in the one-kidney dogs, the average renal oxygen consumption in experiments without urea administration was 4.4 cc. per minute; in experiments with urea it was 4.7. In the two-kidney dogs the average renal oxygen consumption in experiments without urea administration was 5.8 cc. per minute, while when urea excretion was increased by giving large amounts of urea or meat the oxygen consumption averaged 4.6 cc. per minute.

The apparent significance of these results is that not only the work directly involved in concentrating the urine, but also whatever processes are indirectly connected with it, taken together, consume so little of the total oxygen absorbed by the organ that maximal variations in the excretory work are without measurable influence on its total oxygen consumption. The overwhelmingly greater part of the energy produced must be utilized by the kidney for its own internal cellular processes not related to the external work which the organ is performing. In this respect the kidney affords a contrast to muscle.

Borsook and Winegarden (1931) have reported experiments in which the total oxygen consumption of human subjects was determined before and after feeding about 0.25 gram of urea per kilo body weight, much less than was given in some of our experiments. Borsook and Winegarden observed increases in the oxygen consumption of their subjects, which they interpreted as a measure of the increase in excretory work of the kidneys. In the light of our results obtained directly from the kidney, it appears that such an interpretation is impossible, and that the increases in oxygen consumption observed by Borsook and Winegarden must have been either fortuitous or due to stimulation of the general, extra-renal metabolism of the subjects.

DISCUSSION OF RESULTS IN CONNECTION WITH THEORIES OF THE MODE OF URINE FORMATION. The field has been divided between two theories, a modification of the filtration-reabsorption theory of Ludwig and of Cushny (1926), which has received strong experimental support from the work of Richards (1922, 1929, 1933) and of Homer Smith (1932) and their collaborators, and the direct excretion theory, the arguments for which have been presented by Bensley and Steen (1928) and by Volhard (1931).

The *filtration-reabsorption theory* assumes that water and crystalloid products of the urine leave the blood in the glomeruli, where an ultra-filtrate of the blood is formed, containing the diffusible substances in the same concentrations as in the blood plasma. The deviations which these constituents show in the bladder urine from the proportions they hold to each other and to water in the blood plasma are, according to this theory, caused by the activity of the tubular epithelium, which reabsorbs most of the water (in mammals usually 97 per cent or more) from the glomerular filtrate, and different proportions of the different solids. Richards (1929) with Wearn (1925) and Bordley, Walker, and Reisinger (1933) has shown, by beautiful ultramicrochemical technique, that the glomerular filtrate of the frog is in fact an ultrafiltrate of the blood plasma, and contains the same concentrations of urea, uric acid, chloride, inorganic phosphate, and sugar. Jolliffe, Shannon and Smith (1932 a, b, c) and Smith, Shannon, and Jolliffe (1932) provided evidence which supports the filtration-reabsorption theory, with modification in the case of creatinine. They found that xylose,

sucrose, and raffinose were all excreted with the same clearance values by normal dogs. Urea clearance was consistently about 70 per cent as great. Creatinine showed clearances 15 to 40 per cent higher than xylose; this

TABLE 9

*Summary of previous data on oxygen consumption by the dog's kidney**

AUTHOR	DATE	PREPARATION OF ANIMAL*	OXYGEN CONSUMED PER MINUTE BY ONE KIDNEY					
			Total			Per gram kidney		
			Max.	Min.	Mean	Max.	Min.	Mean
			cc.	cc.	cc.	cc.	cc.	cc.
Barcroft and Brodie	1904–5	Chloroform anesthesia and evisceration	3.35	0.53	1.56			
Barcroft and Brodie	1905–6	Chloroform anesthesia and evisceration	4.35	0.53	1.44	0.075	0.008	0.029
Bainbridge and Evans	1914	Heart-lung-kidney preparation				0.046	0.040	0.043
Fee and Hemingway	1928	Heart-lung-kidney preparation				0.20	0.03	
Hayman and Schmidt	1928	Sodium phenobarbital and operation to expose vessels				0.113	0.009	
Glaser, Laszlo and Schürmeyer	1932	Various anesthetics. Operation to expose vessels				0.202	0.003	0.070
Our results with 1-kidney dogs		1 kidney explanted, other removed. Animal normal	9.5	1.1	4.5	0.29	0.04	0.15
Our results with 2-kidney dogs. Flow estimated through 1-kidney		1 kidney explanted, other *in situ*. Animal normal	6.6	1.0	3.1	0.14	0.03	0.08

* For methods of measuring renal blood flow for calculation of O_2 consumption, see table 8.

behavior, together with the fact that aglomerular fish were found capable of excreting creatinine, indicated the probability that the extra 15 to 40 per cent of creatinine is excreted by the tubules. Glucose, of course, is not

excreted by the normal dog, reabsorption in the tubules being complete. Treatment with phlorizin apparently abolished the ability of the tubules both to reabsorb glucose and to excrete creatinine, for it caused the clearances of both these substances to approximate the xylose clearance. Cope (1931 and 1933) working with rabbits, has obtained similar results, except that in these animals sucrose behaved like creatinine. The xylose clearance is evidently a most significant value: Smith and his collaborators regard it as the true glomerular filtrate, from 15 to 40 per cent less than the creatinine clearance calculated as the glomerular filtrate by Rehberg (1926).

The *direct excretion theory*, contrary to the above, assumes that, while the water may be filtered in the glomeruli with a small proportion of the solids which appear in the urine, the much greater concentration of certain solids in urine than in blood is caused by the activity of the tubular cells in taking these solids from the blood and passing them into the glomerular fluid as it passes down the tubules. Marshall (1931) in experiments on dogs and Chambers and Kempton (1933) with tissue cultures of chicks' renal tubules appear to have shown that phenol red thus enters the urine through the tubular lining, but similar demonstration for the naturally occurring urine solids is lacking. Oliver and Shevky (1929) working with isolated perfused frog's kidney, concluded that phenol red was excreted by the glomeruli. But they found neutral red excreted by the tubules, and supported the principle of possible tubular excretion.

Compatibility of present results with the filtration-reabsorption theory. This compatibility is most easily presented by fitting our results into an hypothetical description of urine formation according to the theory. For simplicity of expression, and to avoid continual reservations, we shall outline the entirely tentative explanation of the excretory processes in the following paragraphs as though the filtration-reabsorption theory and its corollaries were demonstrated truths.

The blood passing through the glomeruli pours its ultra-filtrate into the capsules. The force which drives the filtrate out of the blood is the blood pressure in the glomerular capillaries. The opposing force which limits the amount of filtration is the osmotic attraction of the plasma proteins for water, plus any back pressure that may exist in the fluid in the capsule.

When the blood flow in the glomeruli increases from opening of more capillaries in the tufts, as observed by Richards, each freshly opened capillary contributes its quota to an increase in the rate of filtration. Hence the rate of urea excretion, expressed in the urea clearance, increases in proportion to the renal blood flow, as observed in our experiments.

Since the plasma protein osmotic pressure is nearly constant, and the

[5] If, however, one assumes with Rehberg that the clearance of creatinine, rather than xylose, is equal to the glomerular filtrate, the reabsorbed urea is calculated to be about 50 per cent of the filtered urea, instead of 30 per cent.

glomerular blood pressure also presumably so, the proportion of blood water passing into the glomerular filtrate when these forces balance is nearly constant, and likewise the similar proportion of blood urea that passes with this water. Whether the concentration of urea in the blood is high or low makes no difference with the percentage of blood urea removed in the glomeruli, because the urea is filtered out with the water in which it is dissolved. If 13 per cent of the blood water is filtered (*vide infra*), 13 per cent of the blood urea goes with it. Hence we derive what appears to be the simplest explanation for the observed fact that the *percentage* removal of urea from the blood is the same, whether the blood urea concentration is high or low.

From the glomeruli the filtrate passes into the tubules, where the greater part (usually 97 to 99 per cent) of the filtrate water is reabsorbed by the tubular epithelium and passed back into the blood, with a minor proportion of the filtrate urea, about 30 per cent, to judge from comparison with the xylose clearances of Jolliffe, Shannon and Smith (1932b).[5] This process of reabsorption involves accomplishment of external work by the tubular cells. They must withdraw water from the filtrate, pass the water back into the blood, and remain relatively impermeable to passage of urea with the water, despite the fact that toward the end of the process urea may become 100 times more concentrated in the filtrate than it is in the blood. The procedure requires that the cells act as force-pumps to drive water out of the filtrate against a heavy head of osmotic pressure. Of the mechanism by which the cells thus act we know nothing, except that it must involve work by the cells, done by means of energy produced in them, presumably by fuel combustion. And we have seen that a reflex disturbance produced while puncturing the renal vein may cause the cells to stop this work. Thereupon the tubular lining becomes an ordinary permeable membrane, and permits free diffusion of urea back into the blood (fig. 3). Even when the kidney is functioning normally, tubular impermeability to urea is not perfect, and about 30 per cent of the filtered urea passes back with reabsorbed water. On this basis one would estimate therefore that the extraction of the average 9 per cent of the arterial blood urea by the kidney is accomplished by filtering about 13 per cent of the blood urea in the glomeruli, and that of the 13 per cent 4 is reabsorbed, lowering the percentage extraction by the kidney to the 9 per cent observed. The usual variation between 6 and 12 in the percentage extraction of urea may be attributable chiefly to variations in reabsorption. Since reabsorption is controlled by cellular activity, it may be expected to be subject to more variability than the process of glomerular filtration, the extent of which depends on the balancing of relatively constant physical forces.

When reabsorption of water from the filtrate exceeds a certain proportion, the concentration of urea in the filtrate becomes so great that it begins to

break through the relative impermeability of the tubular epithelium and to pass back into the blood in increasing proportions. Then the percentage urea extraction, and in consequence the urea clearance, begins to decrease. This occurs when water reabsorption becomes sufficient to reduce the urine flow below the "augmentation limit" of Austin, Stillman, and Van Slyke (1921).

The momentary passage of urea from the kidney back into the blood observed in several of our experiments may be due to reflex paralysis of the tubular cells. When it occurs the tubular lining becomes a membrane without specific impermeability towards urea, and permits the latter to pass back into the blood in accordance with the ordinary laws of diffusion. The occurrence of the phenomenon in our experiments indicates the readiness with which tubular impermeability to urea may be decreased or abolished. It appears possible that such decrease may have caused the temporary low urea excreting power observed by Rehberg (1932) in a case of partial ureteral obstruction by renal calculus, in which the excretion of creatinine remained more nearly normal.

Compatibility of results with the direct excretion theory. It is possible also to build a picture of the observed phenomena on the assumptions that all the water filtered in the glomeruli passes into the ureter, without loss by tubular reabsorption. Under these conditions the urea concentrations in urine 50 or more times the concentration in blood would be attained by extrusion of urea with little or no water from the blood through the tubular epithelium into the filtrate as the latter passes down the tubules. In this process, as in that of the filtration-reabsorption theory, the active thermodynamic work must be done by the tubular cells. In this case, however, they do it, not by pumping water against the force of osmotic pressure from the filtrate back into the blood, but by forcing urea in the opposite direction from blood to filtrate. The forcing of urea from blood to filtrate here assumed is, however, against the force of osmosis, as is the opposite pumping of water assumed in the filtration-reabsorption theory. Hence a similar amount of work would be required in either process.

Relative applicabilities of the two theories to present results on urea and water excretion. At present a definite decision between the two theories of renal excretion in the higher mammals can not be made. The fact or facts necessary for such a decision have not been discovered. All which have thus far been found can be made to fit into either explanation. Nevertheless, it must be admitted that the brilliant work of Richards and his collaborators in proving that the glomerular fluid of the frog is a true ultrafiltrate of the blood has demonstrated the possibility that the filtration-reabsorption theory is adequate, and it furthermore is difficult to explain the results of Homer Smith and his collaborators on any other basis without

assuming improbable coincidences. Also the facts presented in this paper appear to be most easily explained by the filtration-reabsorption theory.

As seen from figure 8, there is no relation between the rates of renal blood flow and urine flow. If the urine water were removed from the blood solely by direct filtration, without reabsorption, one would expect some parallelism between blood flow and urine flow, as there is between blood flow and the urea clearance (fig. 5).

It appears somewhat easier to explain also the relative constancy of the percentage urea extraction by means of the filtration-reabsorption theory, in which filtration of a constant proportion of the blood water and urea is predictable from fairly constant physical forces controlling the process. According to the direct excretion theory, the removal of urea from the blood would depend directly upon the specific activity of the tubular cells; one would expect more variability in such activity than in the filtration process. The latter appears to offer the more satisfying explanation of the relative constancy of the percentage urea removal, and the maintenance of that constancy in the presence of great changes in blood urea content. The great variability in urine volume is more what one would expect of cellular activity, with its probable responsiveness to stimuli. The observed relative constancy of percentage urea extraction and clearance, and the contrasting extreme variability of water output, appear most compatible with the assumption that the urea removal is controlled chiefly by the relatively constant physical forces governing glomerular filtration, while the variable water output is controlled chiefly by cellular activity governing tubular reabsorption.

Causes which may diminish the urea clearance. Whatever theory of excretion is correct, when the volume of blood cleared of urea per minute is diminished, the diminution must result either:

I. From decrease in the volume of blood perfusing the kidneys per minute, or

II. From decrease in the proportion of blood urea removed.

Decreased clearances from both causes have been observed in experiments recorded in this paper.

The physiological and pathological conditions which may produce each of these two effects may, in part at least, be summarized as follows:

I. *Retarded renal circulation.* This may arise from:

a. Closure of an unusually great proportion of the glomerular capillaries by over-stimulus of the normal function noted by Richards (1929).

b. Functional constriction of the afferent arteries of arterioles, the angiospastic constriction believed by Volhard (1931) to be the immediate cause of renal damage in acute nephritis. Reversible.

c. Passive congestion, in cardiac failure or in shock. Reversible.

d. Anatomical occlusion of arterial lumina. Advanced renal disease,

whether initial stages were of the sclerotic or the hemorrhagic type. Irreversible.

e. Destructive occlusion of glomerular capillaries. Advanced renal disease of either type. Irreversible. Van Slyke, Stillman, Möller, Ehrich, et al. (1930) and Hayman and Johnston (1933) have found that decrease of perfusible glomeruli in nephritis parallels the fall in urea clearance.

II. *Decrease in the percentage of urea removed from the renal blood* may result from the following causes, all of which appear to be possibly reversible.

a. *Filtration of a smaller volume of fluid from each cubic centimeter of blood perfusing the glomeruli.* This may result from several causes, as follows:

1. Decreased permeability of the glomerular capillaries. This might so retard filtration that the blood would leave the glomeruli before filtration had reached the extent limited by the opposing pressures which set its ordinary limit. Consequently instead of about 13 per cent of the blood water and urea being filtered, only a fraction of this proportion might pass into the glomerular capsules.

Decreased permeability might occur from thickening of the capillary walls in the tufts, or of the enfolding membrane that surrounds the capillaries, or from collection of protein in the space within or without this membrane.

Whether such decrease in permeability ever plays an important rôle in renal disease we have no data to judge. It may be that the processes of arterial and capillary occlusion cut off the blood flow before decreased capillary permeability becomes important.

2. Decreased blood pressure in the glomerular capillaries. This might result from fall in the general arterial pressure, from constriction of the afferent arteries, or from widening of the efferent ones.

It is known that a decrease in general arterial pressure below about 40 mm. results in anuria, presumably because the pressure in the glomerular capillaries falls too low to push filtrate out against the opposing forces, viz., the osmotic suction of about 25 mm. of mercury exerted by the plasma proteins and whatever hydraulic back pressure exists from the tubular fluid. The decreases in blood pressure which occur in acute cardiac failure and in shock may lower the urea clearance.

Whether, on the other hand, general arterial hypertension in renal disease acts to raise glomerular pressure and compensate for other changes is a question still in dispute. If the hypertension did serve a compensatory purpose, one might expect that fall in blood pressure in a chronic case might cause fall in urea clearance. Such parallelism has not been observed in our clinic (Page, unpublished).

3. Increased back pressure on glomerular filtrate. This must occur when urinary flow is obstructed, from the bladder, the ureter, or by closure

of the tubules with casts or swollen epithelium. This last may be the cause of decreased urea clearance in mercury poisoning.

Partial obstruction, with increased back pressure but not complete stoppage, may, however, decrease the clearance chiefly by increasing reabsorption in the tubules, as will appear below.

Theoretically, it is possible that retarded reabsorption of water in the tubules might also cause a sufficient back pressure to retard glomerular filtration. In such a condition the volume of urine would be multiplied because of the escape into the ureters of the unabsorbed filtrate. One might accordingly expect to find depression of the urea clearance in conditions of great diuresis, if back pressure from lack of reabsorption is likely to cause such depression. However, in the maximal diureses obtained by Austin, Stillman, and Van Slyke (1921) there was no decrease in the urea clearance. It appears unlikely that this factor is often significant.

4. Increased protein content of the blood plasma. If the limit of glomerular filtration is set by approach to a balance between the restraining colloid osmotic suction of the blood plasma and the capillary blood pressure, it would follow that increase of the protein osmotic suction in the plasma would diminish the volume of filtrate, and hence of the urea clearance. And *vice versa*, decrease in plasma protein content would increase filtration. Experimental data on this point are lacking, and the clinical data are ambiguous. In two of the eight cases of degenerative Bright's disease charted by Van Slyke, Stillman, Møller, *et al.* (1930) there were periods in which the urea clearance was markedly above the usual normal level, sometimes by as much as 40 to 80 per cent, and a similar phenomenon was observed in some cases recovering from acute nephritis. The periods of high clearance in some cases corresponded with those of low plasma protein content, but in other cases the high clearance occurred in the presence of normal plasma protein values.

c. Increased reabsorption of urea in the tubules. Results of our experiments in this paper show that passage of urea back from the kidney into the renal blood can occur temporarily, even in normal kidneys, and to such an extent that the renal vein blood urea content exceeds the arterial. A possible, though not demonstrated cause of the reabsorption may be reflex paralysis of the tubular cells. In the degenerative type of nephritis, where tubular disease is most evident, the clearance does not, however, appear to be decreased unless the glomeruli also are involved (Van Slyke, Stillman, Møller, *et al.*, 1930).

Other clinical conditions have been reported nevertheless, in which there is evidence of abnormally great reabsorption. In one case of obstruction Rehberg (1932) found dilute urine, low urea concentration, and relatively normal creatinine clearance. For the differences in the effect on urea and creatinine excretions Rehberg offered the explanation that the tubules

become permeable to reabsorption of urea more readily than to reabsorption of creatinine. The explanation seems reasonable from the fact that creatinine appears normally to be not reabsorbed at all.

Ferro-Luzzi (1933) states that he has encountered cases of tertiary syphilis and various other infections, in which the blood urea was high, up to 150 mgm. of urea per 100 cc., and the urea clearance low, but the creatinine clearance normal. The urea retention he considers therefore to be due to a loss of the ability of the tubular epithelium to prevent reabsorption of urea. He states that in these cases the urea retention is of no prognostic significance. In patients with Bright's disease Hayman, Halsted, and Seyler (1933) found no types in which the creatinine and urea clearances uniformly differed: the peculiarity noted by Ferro-Luzzi apparently occurs rarely, if at all, in genuine Bright's disease.

SUMMARY

1. Simultaneous studies of renal blood flow, of the removal of oxygen and urea from the renal blood by the kidneys, and of the excretion of urea and water have been carried out on normal, unanesthetized dogs in which kidneys had been explanted by the technique of Rhoads, so that blood from the renal vein could be obtained by needle puncture through the skin. The renal blood flow was calculated from the rate of urea excretion and the decrease in blood urea content which occurred as the blood passed the kidneys. The results apparently afford the first data on the proportion of urea removed from the blood, the blood flow, and the oxygen consumption in the kidneys, which have been obtained without operation or anesthesia.

2. The kidneys of the dogs removed usually 6 to 12 per cent of the urea from the blood which perfused them. This percentage was independent of the blood urea concentration within such wide ranges as 8 to 140 mgm. of urea nitrogen per 100 cc. It is this constancy in percentage urea removal which makes the urea clearance independent of the blood urea concentration.

3. The renal blood flow varied from 2 to 10 cc. per gram of kidney per minute.

4. *The normal spontaneous variations in the urea clearance paralleled chiefly variations in renal blood flow*, the effect of variations in the percentage of urea removed from the blood being less important.

5. In occasional experiments, however, the removal of urea from the blood by the kidney was momentarily reversed, and urea previously concentrated in the kidney diffused back into the blood. This behavior apparently lasted for only a few minutes at each occurrence. A possible explanation is that trauma during needling of the renal vein caused a reflex paralysis of the tubular cells, which lost their relative impermeability to diffusion of urea from the tubular lumina back into the blood. The occur-

rence of the phenomenon suggests that such reabsorption of unusual proportions of urea may, under unusual physiological or pathological conditions, become a cause of diminished urea clearance, even through the usual cause is retarded renal blood flow.

6. Removal of one kidney was followed by an increase in the blood flow, oxygen consumption, and urea clearance of the remaining kidney. The increase in most cases appeared to reach its maximum within a month. The average increase in blood flow was 68 per cent, in oxygen consumption 81 per cent, and in urea clearance 43 per cent, of the pre-operative values.

7. No relation of renal blood flow or renal oxygen consumption could be found to the work of the kidney in excreting either water or urea at greatly varying rates. It appears that neither the excretory work nor the processes directly connected with it control the oxygen consumption of the kidney, which must be governed by the energy requirements of the non-excretory processes in the organ. This conclusion is explicable by the fact that, as calculated by Brodie, Barcroft, and others, the thermodynamic work ordinarily done by the kidney in excretion is less than 1 per cent of the energy furnished by the respiration of the organ.

8. Renal oxygen consumption and blood flow tend to run parallel in their variations. Presumably both respond to similar metabolic demands of the kidney.

9. The blood in the renal vein is usually more than 85 per cent oxygenated, indicating that the tissues in the kidney are kept under higher oxygen tension than in most other organs.

10. Applications of the results to the theory of renal excretion, and to explanation of the mechanisms of physiological and pathological variations in urea clearance are discussed.

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THE LIBERATION OF ADRENERGIC AND CHOLINERGIC SUBSTANCES IN THE SUBMAXILLARY GLAND

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The submaxillary gland receives its innervation through two distinct nerve trunks, the sympathetic and the parasympathetic (chorda tympani). Stimulation of either of these nerves results in the production of saliva. In the cat the saliva thus produced contains water, salts, and organic materials in varying but similar proportions. This close similarity has long given special interest to the study of the mechanism of salivary secretion in the cat's submaxillary gland. Recently particular emphasis has been placed upon the possible rôle of neuro-humoral factors in this mechanism.

Babkin, Gibbs and Wolff (1932), Gibbs and Szelöczey (1932), and Babkin, Alley and Stavraky (1932) have shown that stimulation of the chorda tympani, in addition to the local secretory and vasodilator effects, results in the liberation into the blood stream of a chemical agent which under specified conditions induces a fall in the systemic arterial pressure and secretion of saliva in the opposite gland. It has been shown in addition to possess other cholinergic properties. Similar observations have been made independently by Beznák (1932), who has shown by means of cross-circulation experiments that blood coming from a gland activated by chorda stimulation causes salivary secretion in a second animal and that such blood is also effective in reducing the contractility of the isolated frog heart. The latter finding has also been reported by Henderson and Roepke (1933). Furthermore, Secker (1934) has demonstrated that the saliva produced by chorda stimulation of the cat's submaxillary gland has acetylcholine-like properties.

Besides having an easily calibrated external secretion controlled by specific nerves, the submaxillary gland has a further advantage in relation to studies of this type in that the blood flow can be readily measured. In the present experiments we have been concerned with the analysis of the mechanism of secretion and changes in blood flow, with special reference to the nature of the chemical agent liberated on stimulation of sympathetic nerve fibers.

METHODS. All the observations have been made on cats, anesthetized

with "Dial-Ciba" (di-allyl-barbituric acid, made up with urethane and a small amount of ethyl carbamate to insure solution), injected intraperitoneally in amounts of from 0.050 to 0.065 gram per kilo. The lingual nerve trunk, containing the fibers of the chorda tympani, and the cervical sympathetic nerve were isolated, severed from the central nervous system and arranged for stimulation. Destruction of a majority of the sympathetic branches other than those going to the gland was accomplished by cutting cephalad to the superior cervical ganglion and crushing the larger arterial branches with a hemostat. A control on the success of this procedure was furnished by the absence of blood pressure changes or evidence of a chemical substance when the venous outflow from the submaxillary gland was prevented from returning to the general circulation. In those experiments in which the opposite salivary gland or the nictitating membrane was used as an indicator of the presence of a chemical agent, the superior cervical ganglion of that side was aseptically removed from 4 to 8 days previously.

In order to measure the blood flow through the submaxillary gland a cannula was inserted through a side vein so that its tip was inside the external jugular. With the exception of the vein coming from the gland all other contributory veins were tied off. When it was desired to record the blood flow a clip was placed centrally on the jugular so that the entire venous return from the gland passed to the exterior by means of the cannula, making possible the recording of individual drops through a signal magnet writing on the kymograph drum. In these experiments the coagulation of the blood was prevented by the intravenous injection of 70 mgm. per kilo of heparin (H. W. & D., Baltimore) or "Novirudin" (Norgine A. G. Prag) in doses of 120 mgm. per kilo.

Hypodermic needles with rounded tips were employed for the cannulation of the salivary ducts. By means of the drop recorder described by Gibbs (1927) the drops of saliva were directly recorded on the kymograph tracing.

In certain experiments we utilized the denervated nictitating membrane on the opposite side as an indicator of humorally transmitted effects of salivary secretion. Following Rosenblueth and Cannon (1932), we have found this organ extremely sensitive to adrenin-like substances. The membrane was directly connected by means of light silk thread to a heart lever recording on the smoked surface.

The blood pressure of the femoral artery was recorded by means of a mercury manometer. A second cannula was inserted in the femoral vein of the opposite side for the injection of various substances used in the analysis of the autonomic effects. Details of these will be given in connection with the presentation of the data.

RESULTS. At the outset it may be stated that evidence has been obtained of the liberation of substances into the blood stream upon stimula-

tion of the nerves to the submaxillary gland. In the case of the chorda tympani this substance has, to use the terminology proposed by Dale (1933), cholinergic properties, while sympathetic stimulation confers adrenergic properties on the blood.

Evidence from salivary secretion. Since the pioneer investigations of Langley (1878) it has been known that the secretory mechanisms of the sympathetic and parasympathetic fibers are not identical. This fact is well illustrated by the action of atropine which, in properly selected doses, blocks the effects on the submaxillary gland of chorda tympani stimulation without influencing the effects of cervical sympathetic stimulation. Physostigmine greatly enhances salivary secretion induced by stimulation of the chorda tympani or the injection of acetylcholine. Similarly, cocaine increases the effects of sympathetic stimulation and of epinephrine. These facts have long been known but as far as we are aware have not been considered in relation to the identification of the chemical substances concerned in neuro-secretory activity of the salivary glands.

In the present experiments a comparison has been made of the amount of saliva secreted in response to uniform periods of sympathetic and parasympathetic stimulation before and after the injection of physostigmine or cocaine. The stimulus consisted in condenser discharges (1 mfd.) of constant intensity and frequency (30 per sec.) applied for uniform periods of time, usually 15 seconds. After establishing control values for salivary secretion, physostigmine (0.25 to 1.0 mgm.) or cocaine (5 to 15 mgm.) was injected intravenously.

The specific effects of physostigmine or cocaine were uniformly obtained; that is, physostigmine caused an increase in the response to chorda stimulation without appreciable effect on the sympathetic secretion, while cocaine resulted in a small increase in the effectiveness of sympathetic stimulation without influencing that of the chorda. In only one experiment (12/7/33 b) was there a small increase in sympathetic secretion following physostigmine, but this increase is probably not significant or might be related to the fact that this animal had been given cocaine previously. The results from 8 experiments in terms of the total number of drops of saliva are summarized in table 1. The figures in parentheses in every case give the number of observations for which the preceding value is the average. These differences are further emphasized by the action of ergotoxin in abolishing sympathetic effects alone and by the specificity of atropine in abolishing salivary effects that follow parasympathetic stimulation.

In the case of chorda stimulation it has been shown that a substance is carried in the blood stream which in the presence of physostigmine may cause secretion in the opposite denervated salivary gland (Beznák, 1932; Babkin, Alley and Stavraky, 1932). In a few experiments we have attempted to show the same thing for sympathetic stimulation. The

effects at best have been small and inconstant. However, the method is not well adapted for the demonstration of chemical agents arising from stimulation of the sympathetics, since the submaxillary gland has a high threshold to adrenergic agents; relatively enormous doses of epinephrine, for example, are required to produce a few drops of saliva.

These results indicate the elaboration of two distinct and specific substances corresponding to the double innervation. The parasympathetic substance resembles acetylcholine, while that resulting from sympathetic stimulation bears a similar relationship to adrenin.

Evidence from contractions of the nictitating membrane. In this group of experiments the sympathectomized nictitating membrane was used as the test object for possible adrenergic substances coming from the submaxillary gland by way of the blood stream. This organ has been shown by Rosen-

TABLE 1

Effect of physostigmine and cocaine on secretion of saliva on chorda tympani and cervical sympathetic stimulation

EXPERIMENT	CHORDA STIMULATION				SYMPATHETIC STIMULATION				SENSITIZING AGENT
	Before		After		Before		After		
11/18/33	20	(2)	45	(2)	4	(2)	4	(2)	Physostigmine
12/ 5/33	4	(4)	10	(6)	4	(4)	2	(5)	Physostigmine
12/ 7/33 b	3	(6)	14	(4)	5	(7)	8	(4)	Physostigmine
12/19/33	5	(2)	25	(2)	4.5	(4)	3	(3)	Physostigmine
12/ 7/33 a	2.5	(5)	3	(6)	2.5	(5)	5	(7)	Cocaine
12/12/33	2	(2)	1	(4)	1	(2)	2	(4)	Cocaine
12/14/33	2	(4)	2	(4)	2	(1)	3	(1)	Cocaine
2/24/34	16	(2)	12	(2)	4	(2)	5.5	(4)	Cocaine

blueth and Cannon (1932) to respond by contraction to agents elaborated during the stimulation of sympathetic fibers innervating smooth muscle. It is perhaps second only to the denervated heart in its sensitiveness to injected adrenin. It proved to be a simple matter to demonstrate the contraction of the nictitating membrane following stimulation of the sympathetic fibers supplying the submaxillary gland on the opposite side of the animal. A record from such an experiment is reproduced in figure 1. In certain instances positive effects were obtained without the use of an augmentor agent. However, the response was more readily obtained following cocaine administration. This was given in 10 mgm. amounts, intravenously, slowly administered and repeated once or twice, if necessary, until a good contraction on stimulation of the sympathetic nerve to the opposite gland was obtained (usually about 20 mgm.). Through this means positive results were obtained repeatedly in all of the 7 cats tested.

Since there always existed the possibility that afferent impulses were set up, thus reflexly causing secretion of the adrenal gland, or that the chemical agent causing the membrane to contract resulted from stimulation of sympathetic fibers other than those going to the gland (a possibility arising from the technical difficulty in securing complete destruction of these fibers), every experiment was controlled by trials in which the venous return from

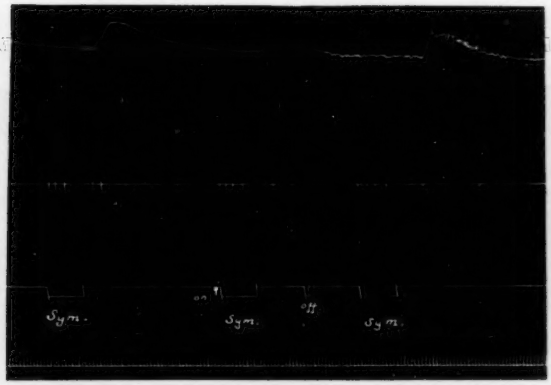

Fig. 1

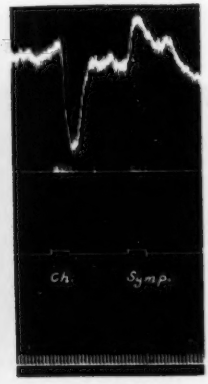

Fig. 2

Fig. 1. Effect on right nictitating membrane (sympathectomized) of stimulation of sympathetic to left submaxillary gland. (Cocaine previously given intravenously.)

Top line—Contraction of right nictitating membrane.

Second line—Secretion in drops of saliva from left submaxillary gland.

Third line—Stimulus signal: *Sym.* = stimulation of sympathetic to left submaxillary gland; *on* = venous outflow from left submaxillary gland blocked with clip; *off* = clip removed from venous outflow.

Bottom line—Time in tenths of a minute.

Fig. 2. Effect on systemic arterial pressure of stimulation of chorda tympani and of sympathetic to left submaxillary gland. (Physostigmine and cocaine previously given intravenously.)

Top line—Arterial pressure recorded from cannula in the left carotid artery.

Second line—Secretion in drops of saliva from left submaxillary gland.

Third line—Stimulus signal: *Ch.* = stimulation of chorda tympani to left submaxillary gland; *Symp.* = stimulation of sympathetic to same gland.

Bottom line—Zero blood pressure and time in tenths of a minute.

the gland was prevented from entering the general circulation. Usually under these conditions no effect whatever was produced on the nictitating membrane. In a few instances, however, as in figure 1, there was a slight contraction, representing but a small fraction of the response obtained with the venous return from the gland intact. This presumably represents a spread of sympathetic impulses to neighboring structures or the escape of

the sympathetic substance into the general circulation through undiscovered and hence unligated venous channels. Commonly in properly sensitized cats there was some spontaneous activity of the nictitating membrane, such as that shown in figure 3. This, however, did not interfere with the recording of the response from distant sympathetic stimulation, since contractions arising from the latter source were much greater.

In a typical experiment contraction of the nictitating membrane took place in from 15 to 45 seconds following the application of the stimulus to the cervical sympathetic trunk innervating the submaxillary gland on the

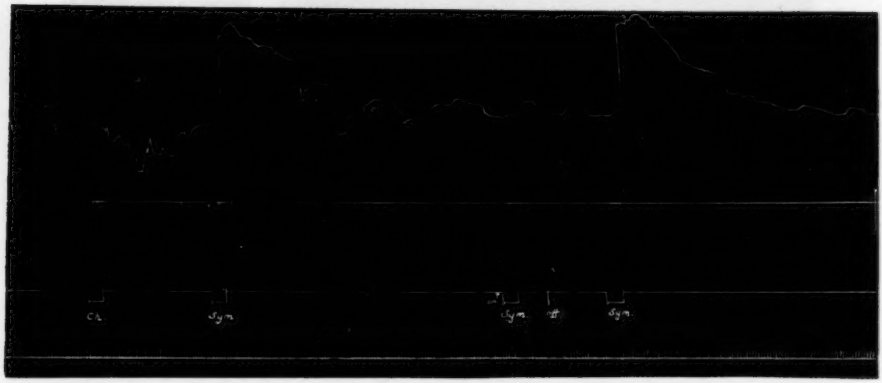

Fig. 3. Effect on right nictitating membrane (sympathectomized) of stimulation of chorda tympani and of sympathetic to left submaxillary gland. (Cocaine previously given intravenously.)

Top line—Contraction of right nictitating membrane.

Second line—Secretion in drops of saliva from left submaxillary gland.

Third line—Stimulus signal: *Ch.* = stimulation of chorda tympani to left submaxillary gland; *Sym.* = stimulation of sympathetic to the same gland; *on* = venous outflow from left submaxillary gland blocked with clip; *off* = clip removed from venous outflow.

Bottom line—Time in tenths of a minute.

opposite side. The phase of contraction was comparatively rapid and was followed by slow relaxation over a period of several minutes. Stimulation of the chorda tympani produced no effect whatever on the membrane. Such an experiment is reproduced in figure 3, which also shows the complete absence of a response when the control procedure is employed of blocking the return flow of blood from the gland during the period of stimulation. The fact that contraction of the nictitating membrane did not occur following chorda stimulation, which is known to produce a cholinergic substance, makes it extremely improbable that such a substance plays any part in the contraction following stimulation of sympathetic

fibers. However, in view of the recent demonstration of the production of a cholinergic substance in relation to the activity of sympathetic ganglia (Feldberg and Gaddum, 1934) and of the adrenals (Feldberg, Minz and Tsudzimura, 1934), it was deemed desirable to control any possible influence of such a substance on the contraction of the nictitating membrane. This was done in one experiment by the injection of 3 mgm. of atropine sulphate, an amount sufficient to block the salivary effects of chorda stimulation or the depressor action of moderate amounts of acetylcholine. The secretion of saliva resulting from the stimulation of the cervical sympathetic trunk was not appreciably affected and, under these conditions, a good response was still obtainable from the nictitating membrane.

It is clear from these results that a chemical agent is elaborated in connection with the stimulation of the sympathetic nerve fibers to the salivary gland and that the substance has adrenergic properties and thus differs from the substance produced during stimulation of the chorda tympani nerve.

Evidence from general and local circulatory effects. The effects of stimulation of the nerves to the submaxillary gland on the blood vessels have been studied from three different angles: 1, the changes in blood flow in the active gland; 2, changes in blood flow of the gland on the opposite side; and 3, general vasomotor effects as observed in changes in arterial pressure.

Repeated observations have been made in 8 cats on the changes in blood flow through the submaxillary gland during chorda and sympathetic stimulation. The results are similar to those which have been reported by various workers. Stimulation of the chorda tympani nerve brings about a great augmentation of the blood flow, the increase for a 15 second stimulus being from two- to tenfold. The rate quickly rises to a maximum and falls off as soon as the period of stimulation is over. In the case of the sympathetic the effect is opposite: initially there may be a complete cessation of blood flow and it is always much reduced during a short period of stimulation. As soon as the stimulus is discontinued there is a sudden increase in blood flow, giving a rate which for a short time is greater than before the stimulus was applied. Since the vasodilator action of the sympathetic occurs only after the cessation of the stimulus, it probably represents a secondary effect perhaps related to an increased metabolic activity of the gland associated with secretion. This may be the explanation of the vasodilator sympathetic effects reported by Carlson and collaborators (1907) and others. Barcroft (1914), who studied the increased oxygen consumption in relation to the production of saliva, proposed this explanation. He noted that vascular dilatation to cervical sympathetic stimulation disappeared after salivation had been impaired by asphyxiation of the gland. On the other hand, chorda stimulation produced vasodilatation

accompanied by a smaller increase in oxygen consumption. This continued after the gland was atropinized and was attributed to a minimal activity. Recent experiments by Gibbs and Szelöczey (1932) have shown that even after complete paralysis of the secretory mechanism of the gland by atropine the cholinergic agent may still be present in the perfusate following chorda tympani stimulation. This is in keeping with Barcroft's suggestion in showing that nervous stimulation may produce effects within the gland apart from the visible production of saliva. In two of our experiments the secretory response to sympathetic stimulation became less and completely disappeared in the course of successive periods of stimulation. With the disappearance of the secretion, vasodilatation was no longer present, although vasoconstriction during the period of the stimulation was observed as before.

The effects of physostigmine and cocaine on the blood flow through the gland paralleled their effects on salivary production. That is, when the chorda was stimulated after physostigmine the resultant increased blood flow was prolonged. No such influence on the sympathetic vasoconstrictor effect was observed. Following cocaine, on the other hand, slowing of the blood flow accompanying sympathetic stimulation was slightly more apparent but there was no effect on the dilatation mediated by the chorda

On the systemic arterial pressure, stimulation of the chorda and sympathetic nerve fibers to the submaxillary gland produce opposite effects. In the presence of physostigmine chorda stimulation results in a marked fall in blood pressure, as previously described by Babkin, Gibbs and Wolff (1932). Stimulation of the cervical sympathetic, on the other hand, in many experiments resulted in a transitory rise in pressure. Under the conditions of these experiments the latter effect was small and less constant than the results from chorda stimulation, presumably because the blood pressure was high and represented a less sensitive indicator for the sympathetic agent. However, cocaine exaggerated the effect of sympathetic stimulation on the blood pressure. Records from an experiment showing the effects on carotid pressure of sympathetic and parasympathetic activity are given in figure 2. These effects were no longer obtainable when the venous return was blocked, thus furnishing proof that they are mediated by chemical agents carried from the submaxillary gland by the blood stream.

In 2 cats measurements were made on the blood flow of one denervated submaxillary gland during stimulation of the nerves to the other gland. A summary of the results from 28 trials in these two animals is given in table 2. While the changes produced are small, they are definite and correspond in sign to the effects produced on the blood flow of the directly stimulated gland. The results become the more significant when considered in relation to the changes in general blood pressure which, when appreciable alteration took place, were always in a direction which would affect the

blood flow in a manner opposite to that observed. For example, a decreased blood flow in the denervated gland produced by sympathetic stimulation commonly occurred in the face of an accompanying small increase in blood pressure (averaging 5 mm. Hg).

DISCUSSION. The observations described in this paper demonstrate the presence of a chemical agent in the blood coming from the submaxillary gland during stimulation of the cervical sympathetic nerve, which is capable of causing contraction of the denervated nictitating membrane, a decrease in blood flow in the opposite gland, and an increase in arterial pressure. This result is in accord with earlier studies indicating that the activity of sympathetic nerves is accompanied by the liberation of a substance having the general properties of adrenin. Such a substance was first demonstrated by Loewi (1921, 1922) in the heart, by Finkelman (1930) in the gut, by

TABLE 2

Effect on blood flow of contralateral submaxillary gland

SYMPATHETIC (+ COCAINE)					
Trials	Decreased flow	No change	Average decrease	Systemic arterial blood pressure	
				Rise	No change
			per cent	*per cent*	*per cent*
17	11	6	12	90	10
CHORDA (+ PHYSOSTIGMINE)					
Trials	Increased flow	No change	Average increase	Sytemic arterial blood pressure	
				Fall	No change
			per cent	*per cent*	*per cent*
11	7	4	11	83	17

Cannon and Bacq (1931) for various organs containing smooth muscle, and by Bain (1933) for the blood vessels of the tongue. Stimulation of the chorda tympani, on the other hand, results in the elaboration in the gland of a chemical agent having physiological properties similar to acetylcholine, a result falling into line with the effects of parasympathetic stimulation to various other organs.

The submaxillary gland thus presents an interesting condition in receiving its innervation from two distinct sources, each of which elaborates a specific chemical agent, and this by inference indicates two distinct mechanisms involved in the elaboration of saliva. So far as we are aware this and other salivary glands in the cat and possibly the pancreas are the only structures in the body in which both sympathetic and parasympathetic appear to activate the same function, in this case salivary secretion, and do not oppose each other. A priori it would seem improbable that such a double

mechanism should apply to the individual cells of the gland. It is more likely correct to regard the submaxillary gland as a double organ each part of which is under separate nervous control. Strong support for this hypothesis comes from a consideration of the morphology of the gland, it being composed of several very different types of glandular cells. Stormont (1928) and more recently Rawlinson (1933) have reported on the histological changes induced in the submaxillary gland of the cat by electrical stimulation of its nerves. Rawlinson showed that in the case of chorda stimulation the predominant effect was on the alveolar cells in which the nucleus becomes less dense and the cytoplasm more finely granular, whereas stimulation of the cervical sympathetic trunk caused the appearance of cytoplasmic vacuolation in the demilune cells. Besides the data included in the present paper there are various facts in the physiology of the gland which lend support to the hypothesis of intraglandular units controlled by separate mechanisms. In this connection we may mention the fact (Cannon and Cattell, 1916) that the simultaneous stimulation of both nerves results in the algebraic summation of the electric potential changes and secretory responses.

These experiments throw no light on the question of the rôle of the various factors in the normal functioning of the salivary glands. It seems probable, however, that the double nervous control mediated through different peripheral mechanisms has some biological significance.

SUMMARY

1. In the submaxillary gland of the cat, physostigmine results in an increase in the secretory and vasodilator response on stimulation of the chorda tympani nerve without affecting the results of stimulation of the cervical sympathetic trunk. Cocaine increases the secretory and constrictor action resulting from the stimulation of the cervical sympathetic without influencing the chorda response.

2. Stimulation of the cervical sympathetic nerve, after destroying all branches except those going to the submaxillary gland, results in a contraction of the denervated nictitating membrane on the opposite side. This effect is enhanced by the presence of cocaine and is dependent upon the integrity of the return circulation from the stimulated gland.

3. Stimulation of the sympathetic fibers to the gland may cause a slight general vasoconstriction, which is indicated by a rise in arterial pressure and a decreased blood flow in the opposite denervated gland.

4. These results indicate the elaboration of a chemical agent in the submaxillary gland in response to sympathetic nerve stimulation which has adrenergic properties and thus contrasts with the action of the cholinergic agent liberated on stimulation of the parasympathetic fibers.

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